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EVENTS DURING MEIOSIS IN *CHLAMYDOMONAS REINHARDI*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled " Events During Meiosis in *Chlamydomonas reinhardi*" submitted by Reg Storms in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The analysis of serial and individual sections with the electron microscope was used to reveal the fine structural organization of cellular organelles, especially the nuclei and chromosomes, during germination of *Chlamydomonas* zygospores. Stage synchrony and duration estimates were determined in two cultures of germinating zygospores. Using one of these cultures, it was possible to determine when the main meiotic S period occurred. Using the other culture the times of the main meiotic S period and recombination of genetic loci were determined.

Prophase I of meiosis could be subdivided into the classical meiotic stages. The appearance of continuous lateral components and their synapsis occurred during leptotene and zygotene. Synapsis resulted in typical tripartite synaptonemal complexes at pachytene. A pachytene karyotype, using reconstructions from serial sections, revealed 16 individual bivalents plus 4 bivalent arms attached to a common mass of condensed chromatin, yielding a haploid chromosome number of 18-20.

Pachytene was followed by a diffuse stage (diffuse diplotene) when it was difficult to distinguish individual bivalents. The chromatin then condensed during the late prophase I and early metaphase period.

During metaphase I and metaphase II basal bodies were found at the spindle poles. The spindles of both meiotic divisions were intranuclear, although a fenestra was found

at both poles. Both chromosomal and polar microtubules were observed, the chromosomal microtubules attaching to distinct kinetochores at both divisions.

Telophase nuclei contained a single mass of condensed chromatin which quickly decondensed forming an interphase nucleus. The two products of the first meiotic division lay at right angles to one another. This was quickly followed by the second meiotic division.

The two meiotic divisions were shown to take 3.6 hours in both cultures. The first meiotic division takes 3.1 hours while the second takes .5 hours in both cultures. Pachytene was found to be the longest individual stage, taking about 1 hour in both cultures.

The analysis of DNA synthesis during germination in one of the cultures used was monitored by Dr. C.K. Tan. His results indicated that the main DNA synthetic period occurred between 5 and 6 hours after the onset of germination. This is a period just prior to prophase I. The time of the main DNA synthetic period in the other culture also occurred just prior to prophase I.

The DNA synthesis inhibitor FUdR caused an increase in recombination when pulsed at pachytene. This FUdR effect is coincident with the latest time in germination when several DNA synthesis inhibitors affect recombination in *Chlamydomonas*. This provides the only compelling evidence that recombination events are fixed at this time.

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CHAPTER I

INTRODUCTION

An understanding of the process of meiosis is of fundamental importance to eukaryotic genetics since it is during this period that the processes of genetic recombination and haploidization occur. Although our understanding of meiosis has expanded greatly at the biochemical (Stern and Hotta, 1973), cytological (Henderson, 1970; Gillies, 1975a; Westergaard and von Wettstein, 1972; Moses, 1968; Heywood and Magee, 1976) and genetic (Baker *et al*, 1976) levels, a comprehensive understanding of these processes and their interactions is far in the future. One of the main reasons that the interaction of these processes and, in fact, the processes themselves are so poorly understood is that very few organisms, if any, afford the opportunity of studying several of these processes simultaneously.

The study of meiosis and the interrelationship of recombinational (genetic), cytological and biochemical observations requires an experimental system with the following features:

1. large numbers of cells easily obtainable
2. synchronous meiosis
3. known meiotic cytology

4. available genetic markers
5. meiotic mutants (and the possibility of obtaining more)
6. collection of samples from the meiotic population without disturbing the meiotic process in the remaining population

Organisms with these features would enable the collection of samples from the same population simultaneously and at different times for the separate analyses of cytological, recombinational and biochemical events. Such analyses would permit the assignment of recombinational and biochemical events during meiosis to cytological stage. Unfortunately, no organism seems to meet all of the above requirements. Several provide excellent systems for the correlation of cytological events and the time of crossing-over as determined by changes in chiasmata frequency (Henderson, 1970; Whitehouse, 1973). Others have been useful for the correlation of biochemical and cytological events (Stern and Hotta, 1973), while a third group has been useful in the correlation of stage with the time of recombination of genetic markers (Lu, 1970).

So far, the biochemical parameters of meiosis have been worked on mainly by Stern's group (review by Stern and Hotta, 1973) in the genus *Lilium*. *Lilium* is an excellent organism for studying the cytology and biochemistry of meiosis. This is because of the large size of its chromosomes and the availability of techniques for the collection

of synchronized meiocytes. *Lilium* also constitutes a system with a very long meiosis which makes manipulations much easier. However, it has the shortcoming of not having any genetics worked out in it, and due to its inappropriateness for genetic analysis, will not lend itself easily to a rectification of this. It is therefore impossible to look at the process of genetic recombination or analyze meiotic mutants in that system at this time.

Yeast (*Saccharomyces cerevisiae*) provides an excellent system for the study of meiotic recombination due to the large number of genetic markers available (Plischke *et al*, 1976). Yeast is also an excellent system with regards to the ease with which large numbers of cells can be obtained (Haber and Halvorson, 1975). In addition, several meiotic mutants exist (Baker *et al*, 1976) and the methods for isolating others are relatively easy. Yet, it cannot be synchronized to a high degree (Roth and Halvorson, 1969) and the cytological events are not readily distinguishable at the light microscope level, although a recent method developed by Zickler and Olson (1975) does improve fixation of yeast so that typical synaptonemal complexes can be observed during meiosis.

The fungus *Coprinus lagopus*, on the other hand, provides an excellent system for the study of meiosis, meeting, to some extent, all six requirements (Lu and Raju, 1970). But it does not provide the synchrony that *Lilium* provides,nor is it as easily handled for biochemical

analysis.

Drosophila provides excellent synchrony within cysts of the vitillarium although the cytology of meiosis still presents problems (Rasmussen, 1974; Carpenter, 1975b). Several meiotic mutants have been obtained (see Baker *et al*, 1976 for review) and many genetic markers exist, making *Drosophila* a fine system for the analysis of recombination, although tetrad analysis is not possible. However, *Drosophila* does not enable the sampling of a portion of the cells within a cyst for cytological stage while still allowing the rest to proceed through for measurement of cross-over frequencies. It is also very difficult to do biochemical analysis using this organism.

Of the organisms available for the study of meiosis, *Chlamydomonas reinhardi* provides a nearly ideal system for analysis. Of the six requirements listed above as desirable, *Chlamydomonas* probably comes closest to meeting them all. An extensive linkage map (Hastings *et al*, 1965), the ease with which large numbers of cells can be collected (Chiu and Hastings, 1973), and the ability to partition the population without disturbing meiosis already exist. Unfortunately very little is known about the cytology of *Chlamydomonas*. This gap in the understanding of meiosis in *Chlamydomonas* makes it difficult to correlate the recombinational and biochemical events with chromosomal events. A second consequence of this lack of knowledge is that before extrapolating from it to other organisms, one must

determine whether the phenomena concerned are atypical or not.

It will be the objective of this investigation to define the cytology of meiosis in *Chlamydomonas reinhardi* using light and electron microscopy and then define the time and duration of the different stages. This being established, it will then be possible to; correlate the time of the different biochemical events with meiotic stage, compare the meiotic events in this organism with those known to occur in other organisms, and establish the time of meiotic crossing-over by correlating the stage in meiosis when pulses of FUdR modify recombination levels. The establishment of the above will prepare this system for further experiments towards an understanding of meiosis.

1. *Pre-meiotic Stages*

The question whether pairing and/or recombination take place prior to meiotic prophase is still controversial. Different authors (Maguire, 1967, 1972; Grell, 1973; Sherman and Roman, 1963) have either reported the occurrence of pre-meiotic pairing from cytological evidence or have interpreted the sensitivity of recombination at a pre-meiotic stage as indicating pre-meiotic pairing and/or recombination.

Maguire (1967) , studying homologous heterochromatin at pre-meiotic stages and homologous chromosomes at pre-metaphase and anaphase-telophase of the last pre-meiotic

mitosis in maize (Maguire, 1972), concluded that pairing does occur prior to prophase of meiosis. Similarly, Grell (1969), using *Drosophila melanogaster* females, believes that she can alter recombination rates prior to the time of prophase. This sensitive stage is believed to be coincident with the time of pre-meiotic DNA synthesis. She argues that because recombination rates can be altered, recombination must take place at this time, and because recombination takes place at this time, the chromosomes must be paired. A third case supporting this view is the one made by Sherman and Roman (1963) using yeast. They found that transferring diploid yeast from sporulation medium to growth medium early during the sporulation process caused the cells to revert to a mitotic cycle. They also discovered that recombination occurred at a reduced level in these mitotic cells. By comparing the time when transfer to growth medium produced recombinant diploids and the time of DNA synthesis, they concluded that recombination occurred at the pre-meiotic S period and at prophase of meiosis.

Unfortunately, none of these cases provide adequate evidence for the authors' interpretations. Maguire's results were obtained using small and difficult to define heterochromatic regions (John, 1976) and are therefore open to controversy. Another investigation by Palmer (1971), using maize homozygous for the <u>am</u> allele, found that there were no such associations and that the two satellite no. 6

chromosomes were unpaired and randomly distributed at the same stage at which Maguire claimed evidence for associations. The am allele also causes meiosis to be replaced by a synchronous mitosis and although this enables the identification of chromosomes due to their more extended nature, it does not represent a perfect control. It is still possible to argue that because of the am allele, normal pre-meiotic associations are removed. It is significant that other workers have not interpreted Maguire's photographs in the same manner as she has (John, 1976) and that in a system where chromosome identification is possible, no associations are seen (Walters, 1970). The solution to this controversy will await an analysis of serials through nuclei using the electron microscope.

The evidence from *Drosophila* is not compelling because the assignment of crossing-over time is made by guessing at what stage of meiosis the cells were in at the time of treatment, by sampling other *Drosophila* at approximately the same stage. Because the synchrony among larvae is poor and the cytology of meiosis is inadequate (Rasmussen, 1974), the estimation of the time when treatments affect crossing-over is very difficult, at best. In fact, Grell may present the strongest argument against her hypothesis in a recent paper (Day and Grell, 1976) showing that synaptonemal complexes are present in cells at this time. Synaptonemal complexes have typically been shown to occur

during prophase of meiosis (Westergaard and von Wettstein, 1972). Their conclusion that synaptonemal complexes are present prior to prophase comes from autoradiographic evidence that this stage with synaptonemal complexes corresponds to pre-meiotic S period. But although autoradiographic evidence may indicate DNA synthesis, it need not indicate main pre-meiotic DNA synthesis. Several organisms have been shown to have DNA synthesis taking place at prophase (Hotta *et al*, 1966; Flavell and Walker, 1973). Unfortunately, in the *Drosophila* system it is not easy to test more rigorously for DNA synthesis.

Sherman and Roman's interpretation of the yeast result is definitely not the only one. In fact, many other workers using other systems (Lu, 1970; Lawrence, 1965; Hastings, 1964; Chiu and Hastings, 1973; Peacock, 1968; Henderson, 1970) have interpreted such results as indicating that there are events occuring prior to meiosis and recombination itself that are pre-recombinational events which, when perturbed, affect meiotic recombination indirectly.

The most compelling evidence that pre-meiotic pairing is unnecessary comes from Lu and Raju's (1970) study of *Coprinus attramentarius* where the cells fuse at the onset of prophase. In *Lilium* Walters (1970, 1972) has shown that until leptotene, chromosomes are single and separate. Walters' results are particularly compelling due to the fact that the species of *Lilium* used has a very obvious

pre-meiotic condensation stage and chromosomes are not paired at this time. Similar results were obtained in *Melanoplus femur-rubrum* (Church, 1972), in mouse (Hsu *et al*, 1971) and in *Nicotiana otophora* (Burns, 1972).

All the evidence that pairing takes place prior to prophase is thus open to question, while the evidence that pre-meiotic pairing does not take place in some organisms is compelling. It can therefore be concluded that pre-meiotic pairing is not necessary for pairing at meiosis in several unrelated organisms and may not exist at all. If one assumes that the process of meiotic pairing is as universal to meiosis as are other features (Gillies, 1975a) it follows that pre-meiotic pairing is not important.

It should be noted that the above evidence excludes gross pairing at the light microscope or even the electron microscope levels, but there still exists the possibility that pairing at the molecular level is occuring at these early stages in all these organisms except *Coprinus*.

The main pre-meiotic DNA synthetic period is more extended than mitotic DNA synthesis periods in those organisms in which the length of both have been checked (Callan, 1972; Kofman-Alfaro, 1970; Bennett and Smith, 1972; Crone, Levy and Peters, 1965; Bird and Birnstiel, 1971; Coggins and Gall, 1972; Wimber and Prensky, 1963; Ghosal and Mukherjee, 1971). This increase in length appears to be a lack of initiation and elongation sites rather than a

reduced rate of synthesis/site (Callan, 1972). It is not obvious whether the extended nature of synthesis is indicative of a fundamental difference between the pre-meiotic DNA synthetic period and the mitotic S period or not. It could be that the reduced rate is just a reflection of the general trend that "all cell cycle events seem to be extended in meiosis and in tissues which are going through mitotic divisions leading up to meiosis" (Bennett *et al*, 1973).

Hotta *et al* (1966) showed that although the bulk of DNA is synthesized at the pre-meiotic S period, a small portion of the nuclear genome, about 0.3%, is not replicated until zygotene (Hotta and Stern, 1971a). Evidence from the study of *Chlamydomonas* (Chiu and Hastings, 1973) suggests that these regions of delayed replication are involved in recombination, and increasing the amount of unreplicated regions increases the amount of recombination. It has generally been shown that perturbations at the pre-meiotic S phase using heat, irradiation, DNA synthesis inhibitors and protein synthesis inhibitors can alter the levels of recombination and chiasmata frequency (see Whitehouse, 1965; Henderson, 1970 for reviews). Genetic analysis of tetrads from meiosis in several organisms suggests that crossing-over involves chromatids not unreplicated chromosomes (Whitehouse, 1965). The time of the main S period has been shown to precede meiotic prophase, or overlap the early stages slightly (Henderson, 1970; Stern and Hotta, 1973; Lu and Jeng, 1975; Flavell and Walker, 1973)

and as reported earlier, chromosome pairing does not occur until prophase, so it may be assumed that these effects are indirect. The interesting thing about these studies is that they indicate that important pre-recombinational (cross-over) events do take place during the premeiotic S period and these events control the levels of meiotic crossing-over to some extent.

2. *Leptotene*

Leptotene has been defined as the threadlike state of early prophase where each individual homolog appears as a single separate unit, not in association with its homologous partner. At the ultra-structural level the definition can be qualified further to include the presence of discontinuous lateral elements and the absence of any regions of synaptonemal complex formation (Moens, 1973a). Such a definition must be adhered to cautiously due to the possible asynchrony between chromosomes. Reconstructions of *Bombyx mori* oocytes (Rasmussen, 1976) at about leptotene show that one bivalent is completely paired while most other homologs have not yet engaged in pairing.

The lateral component or axial core, when stained with lead citrate and uranyl acetate after fixation in glutaraldehyde and osmium tetroxide, is more electron dense than the nucleoplasm although generally less electron dense than the chromatin itself. Although the lateral component is generally not continuous along the length of the homolog

at this time, there may be telomeric associations with the nuclear membrane.

Parchman and Stern (1968) demonstrated that inhibition of protein synthesis at any time during meiosis causes meiotic arrest. This may not be very significant, since considerable protein synthesis takes place at all stages of meiosis. Another interesting observation is that reported by Parchman and Roth (1971). They found that transferring *Lilium* buds to culture prior to leptotene caused reversion to a mitotic cycle, while transferring them later than leptotene allowed them to proceed normally through meiosis. However, if they were transferred to culture at about the leptotene stage of meiosis or just prior to leptotene, cells proceeded part way through meiosis in an apparently normal fashion. At pachytene, pairing was found to be normal but when the cells entered late prophase and metaphase I, they formed an excess of univalents.

The conclusion drawn from these results is that synaptonemal complex formation is not sufficient for the formation of chiasmata. However, because no genetic markers are available in *Lilium*, Parchman and Roth were not able to show that recombination was similarly affected. From these observations it can also be concluded that a commitment to meiosis is made in at least two parts. The first ensures "normal" pairing and commits *Lilium* to the meiotic

divisions. The second part is required for normal chiasmata formation and subsequent segregation. A failure to complete the second part does not arrest the cells' procedure through meiosis.

3. *Zygotene*

The beginning of chromosome pairing with the accompanying formation of the synaptonemal complex marks the start of this stage of meiosis. Pairing usually begins at the telomeres, which are usually attached to the nuclear membrane (see Gillies,1975a; Westergaard and von Wettstein, 1972 for reviews). Not all organisms show attachment of all chromosome ends to the nuclear membrane. In *Drosophila* (Carpenter, 1975b) chromosome ends were generally located in one half of the nucleus but were not attached to the nuclear membrane. This may, however, simply reflect the time at which cells were fixed. As Rasmussen (1976) has shown in *Bombyx*, early pachytene bivalents show attachment but late pachytene ones do not. Thus if the membrane attachment does facilitate pairing, it may be short lived once this function is completed.

As the two homologs approach each other, the chromatin of each homolog moves to the outside of the forming bivalent. As the distance between homologs approaches 3,000 Å a central element begins to form (Moens, 1973a; Gillies, 1975b). Synapsis, although usually initiated at the ends, does not necessarily proceed in from the ends

but may be initiated at several sites along a bivalent (Counce and Meyer, 1973; Gillies, 1975b).

The formation of the central region as a result of the moving together of homologs is not the only method of synaptonemal complex formation reported. Rasmussen (1974) and von Wettstein (1977) have found evidence for the insertion of a complete central region between the correctly spaced lateral components in *Drosophila* and *Neottiella*, respectively.

Several organisms including yeast, insects, maize and *Lilium* show clustering of chromosome attachments on the nuclear membrane during zygotene and early pachytene (Moens,1973b; Gillies, 1975b ; Moens, 1972; Rasmussen, 1976). This is referred to as the bouquet stage. Gillies (1973) found no pattern of nuclear envelope association at pachytene in maize. This cannot be taken to mean that a bouquet arrangement did not occur at zygotene, as Moens (1973b) and Rasmussen (1976) have shown that the zygotene arrangement becomes randomized as prophase proceeds. The attachment of chromosome ends to the nuclear envelope, the clustering of ends on the nuclear envelope at the pairing stage and the apparent initiation of pairing at the ends in many organisms suggests that pairing is a membrane mediated event.

Several interesting biochemical events take place during zygotene (Hotta and Stern, 1976). Most of the bio-

chemical analysis has been carried out using *Lilium* meiocytes. It has been shown in *Lilium* that approximately 0.3% of the nuclear DNA is not replicated at the pre-meiotic S period but is delayed until zygotene (Hotta *et al*, 1966). This DNA has a higher G&C content than the bulk DNA and is unique in sequence (Stern and Hotta, 1974). DNA synthesis has been shown to be necessary for pairing at pachytene by Roth and Ito (1967) who inhibited DNA synthesis at zygotene and found that the formation of synaptonemal complex was arrested but stretches which had already formed were not affected.

An analysis of meiotic DNA synthesis in rye and wheat by Flavell and Walker (1973) did not demonstrate a discrete type of DNA synthesis at zygotene as was found by Hotta and Stern (1971a) in *Lilium*. It was not obvious if this was because there was no DNA synthesis at this time or that the main pre-meiotic DNA synthetic period overlapped with zygotene in these organisms, or that asynchrony in the population prevented its detection.

The 0.3% of the nuclear DNA which is replicated at zygotene is not ligated to the rest of the DNA prior to the completion of prophase (Hotta and Stern, 1976). This DNA has been shown by autoradiographic analysis to be distributed among all the chromosomes (Ito and Hotta, 1973). Stern and Hotta (1973) suggest that Z-DNA serves as the site for matching of partially condensed homologous chromosomes. Each Z-DNA site would contain its own unique

sequence. These sequences could be trapped in the lateral components and through their association with the lateral components account for longitudinal specificity in chromosome pairing (Stern, Westergaard and von Wettstein, 1975).

Unfortunately, bivalent pairing does not show a stringent requirement for homologous unique sequences. Pairing can occur in haploids (Ting, 1973; Gillies, 1974; Sadasivaiah, 1974). This indicates that unique sequence matching of Z-DNA is not necessary for pachytene pairing. Another indication that pairing may not be due to unique sequence matching is the occurrence of homeologous pairing in wheat when one of the arms of the B-5 chromosome is not present (Riley *et al*, 1966).

It still may be the case that stringent pairing in normal pachytene cells is the result of unique sequence matching and the pairing in haploids and wheat reflects a secondary response requiring less precise alignment.

Chiu and Hastings (1973) suggested that delayed replicons could be the potential sites for recombination. Above it was suggested that they are not the sites of potential recombination but act as sites for the matching of homologs for pairing. The reasons that Hotta and Stern (1976) believe that Z-DNA is not recombination associated are; one, it is not joined to the bulk of the DNA until the end of prophase, and two, "other biochemical events which occur later are very suggestive of recombination and

these events do not require Z-DNA associated gaps to explain meiotic recombination".

4. *Pachytene*

Pachytene is the time when prophase pairing is complete. Analysis using electron microscopy shows that homologs are paired along their entire length and a synaptonemal complex is present between them (see Moses, 1968; Westergaard and von Wettstein, 1972; Gillies, 1975a for reviews). Synaptonemal complexes have been observed in almost every organism in which the fine structure of meiotic prophase has been studied. In organisms covering the complete spectrum of eukaryotes, synaptonemal complexes have been observed during pachytene of meiosis (see reviews by Gillies, 1975a ; Westergaard and von Wettstein, 1972 for a complete up-to-date list).

Although synaptonemal complexes vary in the morphology of lateral and central components from organism to organism, there are almost no exceptions to the general tripartite structure. This structure consists of two lateral elements separated by a central region of lesser electron scattering ability which contains an electron dense central component. Both the lateral and central components are continuous from telomere to telomere in bivalents at pachytene.

The only exceptions to the rule that synaptonemal complexes are present in the tripartite form at pachytene

are a few organisms in which they were not observed and two green algae which had atypical complexes. In *Ulva mutabilis* (Bråten and Nordby, 1973) and *Chlamydomonas reinhardi* (Storms and Hastings, 1977), pairing of homologs was observed during meiosis; this pairing was at the appropriate distance but did not show the typical tripartite structure. Both these green algae lacked a central component.

Interestingly, the red algae *Gonimophyllum, Janczewskia, Levingiella* and *Polycoryne* (Krugens and West, 1972), the diatom *Lithodesmium* (Manton *et al*, 1970a) and the brown algae *Chorda* and *Pylaiella* (Toth and Markey, 1973) have shown typical tripartite synaptonemal complexes. The lack of a central element in the green algae will be discussed in light of evidence to be presented later.

Recently several workers have reported nodes in the central region between the lateral components (Gillies, 1972; Carpenter, 1975a; Zickler, 1973; Byers and Goetsch, 1975; Storms and Hastings, 1977). Carpenter (1975a) has hypothesized that these nodes represent the sites of recombination. This is based on the fact that the number and distribution of nodes is that expected of cross-over events.

Another interesting feature is the dispersal of the bouquet arrangement at pachytene. Moens (1973b) attributes this to the separation of centrioles. He observed the association of the ends of the bivalents with the cent-

rioles. As the centrioles moved to their respective poles, one end of each bivalent followed each centriole. Rasmussen (1976), on the other hand, feels that the formation of nuclear vacuoles at pachytene may be responsible for chromosome dispersal in *Bombyx mori* females. A decision whether or not the dispersal is dependent upon vacuole formation or centriole separation will require more careful observations in these and other organisms.

Several lines of evidence suggest that pachytene is the time of genetic crossing-over. Most of the biochemical evidence has been collected by a group working with *Lilium*. The evidence collected is only suggestive of recombination and is open to several interpretations.

As mentioned earlier, Z-DNA is not fully incorporated into the genome before late prophase. This is consistent with the hypothesis of Chiu and Hastings (1973) which states that the gaps associated with this late replicating DNA are potential sites for recombination. Thus treatment of cells at about the S period with inhibitors of protein synthesis would increase the number of delayed regions and therefore increase recombination. Treatment with DNA synthesis inhibitors would extend the S period enabling a greater number of replicons to be initiated at S and therefore unavailable for recombination events initiated at pachytene. This balance between the number of replicons replicated at prophase and the amount of replication

gains some support from the fact that there are fewer initiation sites during the pre-meiotic S than during the mitotic S in mouse.

Stern and Hotta believe that the Z-DNA is not involved in recombination. Their arguments against Z-DNA involvement are that there are several other events which could account for recombination, namely:

1. special repair type synthesis at pachytene (Hotta and Stern, 1971a)
2. "cyclic" appearance of potential recombination related proteins (Hotta and Stern, 1974; Howell and Stern, 1971)
3. "cyclic" appearance of non Z-DNA associated discontinuities at pachytene (Howell and Stern, 1971)

Hotta and Stern (1976) assume "the sites for crossing-over are provided by a programmed formation of single-stranded nicks". This is based on the observation of Howell and Stern (1971) that single-strand nicks appear in *Lilium* DNA at pachytene and are then sealed. The appearance of these single-strand nicks at pachytene coincide with the appearance of an endonuclease. This endonuclease activity appears during zygotene, reaches a maximum during pachytene and disappears at the termination of pachytene. Also present at this time are kinase and ligase activities, although these are not confined to the zygotene and pachytene stages. Another interesting feature is the

appearance of a protein very much like the gene-32 protein of T_4 which is essential for recombination in T_4. This DNA binding protein peaks during zygotene and falls off by the end of pachytene (Hotta and Stern, 1971b). Coincident with the above is the appearance of repair synthesis during pachytene in *Lilium* (Hotta and Stern, 1971a).

Evidence for repair synthesis at pachytene in other organisms, although not as well substantiated as in *Lilium*, does exist. Mammalian spermatocytes are sensitive to irradiation at pachytene (Kofman-Alfaro and Chandley, 1970). The sensitivity is manifest by an increased incorporation of ^{3}H-thymidine following irradiation at this time. This has been interpreted to mean that there are more repair systems at work at this time than earlier or later. It seems equally likely, however, that this incorporation reflects an increased sensitivity of the DNA to damage at this time, the result being simply more damage and therefore more repair. Lu and Jeng (1975) found that there was ^{32}P incorporated into DNA at pachytene in *Coprinus*. By extrapolation to the *Lilium* system, they have assumed that this is repair synthesis.

However, neither the evidence in favor of Z-DNA associated gaps nor that for gaps induced at pachytene as being the initial sites for recombination is very substantial. In fact, there are several arguments against either

being directly involved in recombination.

The following is a list of arguments against pachytene induced and Z-DNA associated gaps being the initial events leading to recombination. It is not necessary for gaps associated with cross-over events to be induced at pachytene, although Hotta and Stern (1976) come very close to making this assumption. There are far more gaps induced at pachytene than are necessary for the 36 chiasmata that are found on the average in *Lilium*. Smyth and Stern (1973) have shown that P-DNA is synthesized mainly in moderately repeated sequences. This is not what one would expect because fine structure mapping has shown crossing-over to include the structural genes and therefore some unique sequences (Whitehouse and Hastings, 1965). Similarly, there are too many Z-DNA associated gaps, the Z-DNA is not ligated to the bulk DNA until after prophase, and finally, Z-DNA associated gaps are not necessarily the initial events leading to recombination.

However there are several events during meiosis in addition to the nicks necessary for the initiation of recombination which may require the existence of gaps. Pairing, disjunction and terminalization of chiasmata could require the existence of gaps. It is also a requirement of any cross-over event that no matter how the initial nick was produced, endonucleolytic cleavages must be made in order to resolve it.

As mentioned above, Hotta and Stern (1976) argue that nicks induced during pachytene and repair synthesis

at pachytene apparently provide the sites for crossing-over. They then make the extrapolation that the "sites of the nicks are in moderately repeated sequences. These observations exclude Z-DNA and its associated gaps from a direct role in crossing-over". The obvious flaw in their argument is that no evidence exists connecting pachytene induced gaps with recombination.

Lu and Chiu (1976) argue in favor of nicking at pachytene being involved in recombination. They argue that the number of nicks and the length of time they persist are proportional to the amount of recombination. They believe DNA repair synthesis is inhibited by cold treatment. Therefore nicks induced at pachytene will persist for longer periods if the temperature is reduced. Thus the likelihood of their involvement in a recombinational event is increased if the temperature is reduced. Unfortunately, neither repair synthesis nor nicking has been demonstrated in *Coprinus* at pachytene.

Chiu (1973) proposed a similar mechanism to explain the increase in recombination obtained by treating *Chlamydomonas* with different inhibitors during prophase. The only difference between the model for *Chlamydomonas* and the one for *Coprinus* is that the *Chlamydomonas* model proposed an increased persistence of Z-DNA associated gaps due to the inhibitor treatments preventing Z-DNA synthesis.

It seems that there are several interesting and

potentially pertinent facts known about biochemical events at pachytene, yet all correlations with recombination are purely speculative at this time.

Three approaches have been taken to determine the time of crossing-over. One of these assumes a 1:1 correspondence between chiasmata and cross-overs. This assumption has not been verified experimentally, although it has been shown that chiasmata do involve segmental exchange and hence crossing-over (Mather, 1938; Lewis and John, 1963; Whitehouse, 1973). There is also a similarity between chiasmata and crossing-over with respect to their distribution (see Henderson, 1970; Whitehouse, 1965; Taylor, 1967 for reviews). This assumption being made, experiments have attempted to show that perturbations administered to meiocytes affect recombination only if administered at specific times. Making the additional assumption that the latest stage at which an effect can be obtained is the stage when crossing-over occurs, one can estimate the time of crossing-over. Such studies have shown late zygotene to early pachytene to be the latest stage at which chiasma frequencies can be altered by perturbing agents (see Henderson, 1970; Whitehouse, 1965; Taylor, 1967 for reviews). An example of this approach is that of Peacock (1968). Peacock has shown that chiasma formation is sensitive to a temperature treatment at early pachytene in the grasshopper *Goniaea australasiae*.

Another method uses linked markers and performs the perturbations as in the first case. The advantage of this method is that it does not require the assumption that chiasmata are the equivalent of cross-overs. Only one such study has been carried out using an organism with genetic markers, synchrony and adequate cytology. Using *Coprinus*, Raju and Lu (1973a)showed that the latest time at which a temperature treatment can modify recombination is at zygotene to early pachytene. From this they concluded that crossing-over occurs at pachytene.

Several studies have been carried out using *Chlamydomonas reinhardi*. These investigations have shown that the latest time perturbations by irradiation (Lawrence, 1965,1967) and DNA and protein synthesis inhibitors (Lawrence and Davies, 1967; Davies and Lawrence, 1967; Chiu and Hastings 1973) can affect crossing-over is prophase of meiosis. Unfortunately, the cytological observations were inadequate to determine the stage of this prophase effect more precisely. The final report is one concerning yeast carried out by Sherman and Roman (1963) which showed that there were two times, the later one coinciding with what is believed to be prophase, at which recombination could be modified. However, the cytology was very poor and the synchrony also inadequate.

The third method used to determine the time of crossing-over correlates the degree of pairing at pachytene with the amount of crossing-over. A set of experiments

using maize, by Rhoades (1968), has shown that there is an excellent correlation between the two. He used chromosomes carrying deficiencies, translocations and knobs, and compared the degree of cytological pairing with the levels of recombination in heterozygotes and homozygotes. He was able to show a perfect correlation between the amount of crossing-over and the degree of pairing. Rhoades' conclusions, however, are open to two criticisms, 1) that recombination levels may reflect pairing levels simply because pairing levels are a result of the amount of recombination, or they may reflect a component that is common to both, and, 2) that crossing-over takes place after pachytene and pachytene pairing is simply a prerequisite for crossing-over.

5. *Diplotene*

This stage is defined cytologically by the breakdown of pachytene pairing. The bivalents remain intact, being held together by localized regions termed chiasmata. Westergaard and von Wettstein (1972) proposed that synaptonemal complex remnants are the ultrastructural manifestation of chiasmata. Three studies of early diplotene have supported this view (Sotelo et al, 1973; Gillies, 1975b; Solari, 1974). Another report, by Schleiermacher and Schmidt (1973), found similar pieces of complex at mouse diplotene but did not consider it to be chiasmata.

Diplotene chromosomes can vary significantly from

organism to organism in their degree of condensation. Many organisms have very diffuse chromatin at diplotene (Stevenson, 1972; Rossen and Westergaard, 1966; Zickler, 1973; Lu, 1967; Barry, 1969; Dixon, 1973). The chromosomes of this diffuse stage in lower organisms resembles the lampbrush chromosomes of amphibia (Callan, 1963; Gall, 1966; Hess, 1971). This diffuse diplotene stage is usually extended in duration. Other organisms have a much shorter diplotene stage with the chromosomes remaining quite compact. The occurrence of this diffuse type of diplotene is usually associated with active transcription.

It has as yet not been possible to demonstrate a 1:1 correspondence between cross-overs and chiasmata (see review by Henderson, 1970). Correlations with both the exchange of linked markers and chromatid exchanges using autoradiography have failed. The best evidence that chiasmata are cross-over sites involves the correlation of segmental exchange and chiasmata (Mather, 1938; Lewis and John, 1963; Whitehouse,1973). The similarities between chiasmata and cross-overs with regards to distribution, interference, sex limitation and response to environmental and genetic modification also strongly suggest their equivalence (see Henderson, 1970; Taylor, 1967; Whitehouse, 1965 for reviews).

A significant proportion of conversion events do not involve a cross-over (Hurst *et al*, 1972). It would be interesting to know if chiasmata do represent sites of

recombination and if so, whether they represent both conversion events that are associated either with and without recombination of outside markers, or only ones that are associated with cross-overs.

Associated with the nuclei of many meiotic cells are spindle pole bodies (S.P.B.). These S.P.B. may be present throughout the cell cycle or may be synthesized *de novo* in preparation for the first meiotic division. Whether S.P.B. are synthesized *de novo* or are present throughout the cell cycle, duplication of them in preparation for the meiotic divisions takes place during late prophase.

Several types of S.P.B. exist. In most animals centrioles are found at the poles during mitotic and meiotic divisions. In higher plants, no definable S.P.B. are found at the poles of mitotic and meiotic cells. In protists, there are several types of S.P.B. found. Manton *et al* (1970a) described dense flat plates in *Lithodesmium*. Similar structures arise early in prophase of meiosis in *Saccharomyces* and replicate themselves late in prophase (Zickler and Olson, 1975; Moens and Rapport, 1971). S.P.B. of basidiomycetes are globular structures about 0.3 μ in diameter. The globular S.P.B. of *Coprinus lagopus* arise *de novo* late in pachytene and duplicate in late diplotene (Lu, 1967; Lu and Raju, 1970; Raju and Lu, 1973b). Rogers (1973) believes that the S.P.B. of *Coprinus stercorarius* from the pre-meiotic nuclei remain and act as S.P.B. for the first meiotic division.

In *Chlamydomonas reinhardi* (Cavalier-Smith, 1974), it was found that basal bodies were not synthesized until late in prophase and that most cells had not completed this synthesis prior to metaphase I. From this it was concluded that basal bodies did not have a centric role in meiosis.

In *Labyrinthula* (Perkins, 1970; Perkins and Amon, 1969) the *de novo* synthesis of basal bodies (centrioles) takes place during prophase of meiosis and they are found at the poles during both meiotic divisions.

The strangest case is that found in *Lithodesmium* (Manton *et al* , 1970a). Here, as reported earlier, the S.P.B. are polar plates for meiosis I but centrioles are formed for meiosis II when both polar plates and centrioles are found at the spindle poles.

The above may indicate that centrioles are important in some organisms and not in others or it may indicate that the only reason they are present at the poles is to ensure their distribution to both products (Heywood and Magee, 1976).

Recombination or crossing-over is not believed to take place at diplotene or later because neither recombination levels nor chiasmata frequencies have been altered by perturbations later than pachytene (Lu, 1970; Henderson, 1970). However most experiments have used chiasmata as their measurement,so that if chiasmata represent potential

sites for crossing-over, it would be impossible to detect the degree to which this potential is utilized by counting chiasmata.

The only experiments which have correlated specific meiotic prophase stages with treatment effects on recombination frequencies are those of Lu (1970). Here, only temperature and gamma irradiation were used to modify recombination levels. Thus, only one experiment, testing the effect of two agents, on one organism, could have detected late prophase effects.

6. *Diakinesis*

Classically, diakinesis is defined as that stage of meiosis when chiasmata move towards the ends of the bivalents. Little is known about the ultrastructure of chiasmata as they terminalize (Gillies, 1975a).

Nucleoli generally begin to disappear at this stage of meiosis in most higher organisms. In the fungi and algae, a complete range of behavior has been reported. For example, most of the nucleoli fuse during prophase in the flagellate *Barbulanympha*. This mass of nucleolar material remains at the equator during meiosis I and is not incorporated into the nuclei at telophase (Cleveland, 1954). The nucleolus persists throughout the meiotic divisions in the ascomycite *Gelasinospora* (Lu, 1967). In algae, little is known about the dispersal of the nucleolus during meiosis, although a general rule is that dispersal

occurs in preparation for mitotic divisions (Triemer and Brown, 1974).

7. *Later Stages of Meiosis*

Metaphase I of meiosis in higher organisms is characterized by a breakdown of the nuclear membrane. The behavior of the nuclear membrane in lower organisms, particularly the fungi and algae, shows considerable variation. Only two cases have been reported in the literature concerning the behavior of the nuclear membrane during the later stages of meiosis in algae. In *Lithodesmium unduatum* the nuclear envelope breaks down during prophase of both meiotic divisions and is reformed in telophase (Manton *et al*, 1969, 1970a). In the chlorophycean alga *Ulva mutabilis* the nuclear envelope is intact except at the poles where a fenestra is present (Bråten and Nordby, 1973). However, one cannot take results like breakdown of the membrane at face value for it has been shown in *Coprinus radiatus* (Thiekle, 1974) that the membrane remains intact during metaphase, while an earlier report (Lerbs, 1971) had shown nuclear membrane breakdown at this time. Although no cases of both meiotic divisions occurring within the original parent nucleus have been observed in algal systems, Moens and Rapport (1971), using serial sections, have demonstrated that both divisions of meiosis in the yeast *Saccaromyces cerevisiae* take place within the original parent nucleus.

In the algae, although kinetochores have been observed during metaphase of mitosis (Pickett-Heaps, 1970), no report of kinetochores during meiosis was found in the literature. In fact, in the three cases in which the later stages of meiosis were examined - *Lithodesmium* (Manton *et al*, 1970a), *Ulva* (Bråten and Nordby, 1973) and *Chlamydomonas* (Triemer and Brown, 1977) - no recognizable kinetochores were observed, although microtubules did contact the chromosomes of *Lithodesmium* and *Chlamydomonas*.

According to Luykx (1970), there are three distinct types of spindle pole arrangements. These are poles with no apparent microtubule organizing centres (M.T.O.C.), those with centrioles as M.T.O.C. and those with structures other than centrioles as M.T.O.C. It seems that all three types of spindle pole arrangement exist in the algae. In *Ulva*, centrioles move to the poles during mitosis (Løvlie and Bråten, 1970) and meiosis (Bråten and Nordby, 1973) although they remain to one side. In *Chlamydomonas reinhardi*, Cavalier-Smith (1974) reported that centrioles are absent from the poles of most metaphase I cells. This observation should be interpreted cautiously as reports that basal bodies were not associated with the spindle poles during metaphase of mitosis (Johnson and Porter, 1968) have since been shown questionable by Triemer and Brown (1974) in *C. moewusii* and by Coss (1974) in

C. reinhardi using thick serial sections and examining them using high voltage electron microscopy.

There are generally three types of microtubules associated with microtubule organizing centres. There are extranuclear or astral microtubules. These are probably involved in the formation of cleavage furrows. There are polar microtubules; these travel from one pole of the spindle to the other,or pass from one pole to beyond the metaphase plate,but do not attach to any chromosomes. Finally, there are chromosomal microtubules. These pass from the poles to the chromosomes,usually attaching to the chromosomes at discrete kinetochores,although they may attach in a less specific way.

8. *Time of Division Stages, Stage Durations and Degree of Synchrony*

As mentioned earlier, one of the requirements of a system useful for the study of meiosis is a knowledge of the cytology. This would include a knowledge of the stage maxima, stage durations and synchrony. Several methods have been used to determine stage durations during meiosis. One is the autoradiographic method (Bennett *et al*, 1973). Here, stage duration is calculated by seeing how many hours it takes before ^{3}H-thymidine incorporated at the premeiotic S period appears in the cells at each of the different stages. Another method uses the sampling of one portion of an anther at time t followed by the samp-

ling of another portion at a later time t+x. By this method one can estimate the amount of time required for cells to proceed from one stage into some subsequent stage (Bennett *et al*, 1973; Ito and Hotta, 1973). Both these methods have inherent errors which are proportional to the length of the initial reference stage and of the final stage sampled. Neither the autoradiographic method nor the dual sampling method lend themselves to an easy determination of the degree of synchrony.

James (1963) suggested the use of probability paper for analysis of single division bursts. In this method, samples are taken from a culture at different times during the course of meiosis and scored as to stage. One then plots the sigmoid cell increase curve on probability paper. The best fit through the data points is obtained using a type of weighted regression analysis developed by Finney (1971). Once one has the curves for entrance into the given stages or beyond, one can estimate duration, synchrony and time of the maximum for each stage. Using these curves it is also possible to estimate the homogeneity of samples taken at different times.

It is also possible to estimate stage duration by estimating the area under each stage distribution curve. This is done by using histograms centred on the time when samples were taken. The last method has the advantage that one does not assume the stage distribution curves to

be normal. The disadvantage is that maxima and degrees of synchrony can only be obtained by *ad hoc* methods.

CHAPTER II

MATERIALS AND METHODS

Strains:

Strains 137C mating type plus and 137C mating type minus of *Chlamydomonas reinhardi* were obtained from Dr. R. P. Levine. 96% of the zygospores from this cross germinated. Of these, 80 - 90% gave 8 products and 10 - 20% gave 4 products.

Arg-1 mt+ and arg-2 mt-, both arginine-requiring mutants, were originally isolated by Eversole (1966) from the 137C strains. The zygospores from this cross germinated to give about "60%" eights.

Media:

The following media used in this study are described in Levine and Ebersold (1958); Hastings (1964); Chiu (1973) and Sueoka, Chiang and Kates (1967) with some minor modifications.

A. Minimal Medium

Minimal medium is composed of 5% modified Beijernick's solution (Bold, 1942), 5% phosphate buffer, and 0.1% trace element solution in distilled water.

B. Nitrogen-Free Medium

N-free medium has the same composition as minimal medium except that Beijernick's solution was replaced by N-free Beijernick's solution, which does not contain NH_4Cl.

C. TAP Medium (Gorman and Levine, 1965)

TAP medium contains:

Tris (hydroxymethyl) Amino methane	2.42 gms
Beijernick's solution	50 ml
Trace element solution	1 ml
1 M KH_2PO_4 (pH 7.4)	1 ml

Distilled water to 1 litre. Finally 1 ml of glacial acetic acid was added.

Note: For the growth of arginine-requiring mutants, TAP medium is supplemented with 0.4% of casein amino acids (TA medium). Solid media were prepared by adding 1.5% or 4% of agar to the above liquid media.

D. 3/10 HSM (High salt media) (Sueoka *et al*, 1967)

NH_4Cl	0.15 gms
$MgSO_4 \cdot 7H_2O$	0.02 gms
$CaCl_2 \cdot 2H_2O$	0.01 gms
K_2HPO_4	0.94 gms
KH_2PO_4	0.47 gms
Trace element solution	1 ml
Distilled H_2O	1 litre

The composition of solutions used above is as follows:

Beijernick's solution

NH_4Cl	8 gms
$MgSO_4 \cdot 7H_2O$	2 gms
$CaCl_2 \cdot 2H_2O$	1 gm
Distilled H_2O	1 litre

Phosphate buffer

K_2HPO_4	14.34 gms
KH_2PO_4	7.26 gms
Distilled H_2O	1 litre

Trace element solution

EDTA	50 gms
$ZnSO_4 \cdot 7H_2O$	22 gms
H_3BO_3	11.4 gms
$MnCl_2 \cdot 4H_2O$	5.06 gms
$FeSO_4 \cdot 7H_2O$	4.99 gms
$CoCl_2 \cdot 6H_2O$	1.61 gms
$(NH_4)_6MoO_4 \cdot 4H_2O$	1.10 gms
$CuSO_4 \cdot 5H_2O$	1.57 gms

Distilled H_2O to 1 litre and pH adjusted to 6.5 - 6.8 by adding freshly prepared 20% KOH.

Vegetative Culture

A. Asynchronous Culture

Arginine-requiring mutants (mating types + and -) were grown separately on 1.5% TA agar plates under fluorescent lamps.

B. Synchronous Culture

Wild type vegetative cells (mating types + and -) were grown in synchronous cultures at $21^{\circ}C \pm 1^{\circ}$ under 12 h light (4.5 Klx) and 12 h dark regimen in 3/10 HSM (high salt medium) of Sueoka *et al* (1967), with constant shaking, and bubbled with 5% CO_2 in air.

Induction of Gametes

A. Synchronous Gametes

Synchronous gametes were induced from synchronously growing vegetative cells by the nitrogen-withdrawal technique described by Kates and Jones (1964) and Sueoka *et al* (1967). Vegetative cells growing in 3/10 HSM at a final concentration of about 10^6 cells/ml were harvested around the sixth hour of the light period. These were centrifuged and resuspended in N-free medium, and adjusted to a final concentration of about $3x10^6$ cells/ml. The culture conditions were identical to those of synchronous vegetative cultures except that continuous illumination was given.

B. Asynchronous Gametes

Asynchronous gametes were produced from the arginine strains grown on TA plates by transferring to N-free medium and treating them exactly as for synchronous gametes.

Mating of Gametes

Gametes derived from TA plate grown arginine-requiring strains were ready after 6 hours in N-free medium, while wild type gametes derived from synchronous cultures were ready after 16-24 hours.

Zygotes heterozygous for the arginine 1 and arginine 2 loci were produced by mixing equal numbers of gametes of both arginine strains. The mixture was returned to the light without shaking until the completion of mating (approx. 30 minutes). The suspension of young zygotes was then

plated on 4% minimal agar plates.

Wild type zygotes were produced by mixing equal numbers of opposite mating type gametes. These were returned to the light without shaking for about 30 minutes, until the completion of mating. Freshly mated cells were spread on HSM - 4% agar plates.

Maturation of Zygotes

The zygote plates were put under fluorescent lights (4.5 Klx) for 24-45 hours. They were then wrapped in aluminum foil and kept in the dark for at least 7-10 days. At the end of this period the zygotes are ready for germination and are called zygospores.

Germination of Zygospores

Germination of zygospores was induced by a one hour 10 Klx light pulse at 25°C. The plates were then inverted over chloroform vapor for 40-60 seconds to kill unmated cells.

Zygospores were then scraped from the 4% plates and suspended. The zygospores heterozygous for the two arginine loci were resuspended in TAP medium, while the wild type zygospores were suspended in 3/10 HSM.

Both types of zygospores were then cleansed of contaminating unmated cells by differential centrifugation. The pelletted zygospores were resuspended in 3/10 HSM and returned to the light (10 Klx) with constant shaking and bubbling with 5% CO_2 in air.

Genetic Method

The region between arg-1 and arg-2, which are separated by 6 map units on linkage group 1, was chosen for the measurement of recombination. This was done because of the ease with which recombinants can be scored (Hastings, 1964).

Germinating zygospores heterozygous for the two arginine loci were pulsed with the DNA synthesis inhibitor fluorodeoxyuridine (FUdR) at a final concentration of 2mM. Pulses were begun at about 5 hours after the onset of germination. Each pulse lasted 30 minutes. The cells were pelletted, resuspended in fresh TAP medium and then pelletted again and resuspended and spread on 1.5% TAP plates. Each plate received 1000-2000 zygospores. The scoring was carried out using a microscope about one week after plating. Colonies will only grow from those tetrads or octets where recombination occured between the two arginine loci. It is therefore possible to score the frequency of recombination by scoring the zygospores that germinated and grew into green colonies and those that did germinate but did not grow into green colonies.

Cytological Method

For electron microscopy, germinating zygospores were collected at one half or three-quarter hour intervals in a test tube. After centrifugation the media was decanted and 2% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4)

was added. These samples were then placed in the refrigerator at 4°C overnight. The next day the cells and fixative were removed from the refrigerator and allowed to warm to room temperature. Following two washes in 0.1 M phosphate buffer, the pelletted cells and about .2 ml of buffer were mixed and transferred to a piece of filter paper. The cells were allowed to partially dry. Appropriate sized portions were picked up using a tooth pick and transferred to the surface of a 1% agar slab.

Using a Pasteur pipette, a 1% agar solution kept at 40°C was placed over the clump of cells. When the agar hardened, the agar encapsulated sample was trimmed using a razor blade. The sample was then fixed for 2-4 hours in OsO_4 at room temperature.

Following OsO_4 fixation the samples were washed twice in phosphate buffer for 15 minutes each and then dehydrated through a graded alcohol series. The graded ethanol series consisted of 50, 70, 80, 90, 95 and 98.5% steps. Each step lasted 15-20 minutes except the 98.5% step which was repeated three times for at least 20 minutes. Dehydration was followed by three 20 minute baths of propylene oxide. The samples were then added to a 1:1 (v/v) solution of propylene oxide and Araldite epoxy resin (Ladd Research Industries Ltd.) or a 1:1 solution of propylene oxide with Epon (Ladd Research Industries Ltd.). The Araldite consisted of one part Araldite 502 resin to one part dodecenyl succinic anhidride, with 2% dimethyl amino methyl phenol

added to accelerate hardening. The Epon consisted of 45 ml of Epon 812, 30 ml of dodecenyl succinic anhidride, 25 ml of nadic methyl anhidride with 1 ml of dimethyl amino methyl phenol added to accelerate hardening. The samples were then placed uncovered on the counter top for at least 2 days to allow the propylene oxide to evaporate off. The samples were then transferred to rubber moulds filled with the above Araldite or Epon epoxy resin mixtures. The Araldite included samples were polymerized for approximately 24 hours at 50°C and then an additional 48 hours at 60°C. The Epon included samples were placed in the oven at 35°C for a few hours, the temperature was raised to 45°C for about 12 hours and finally, the temperature was raised to 60°C for 48 hours.

Because of the possibility that the cells were impermeable to fixatives and/or embedding material during the early stages of meiosis, the cell wall was removed from some samples. The removal was accomplished by fixing the cell in glutaraldehyde for 10 minutes then vortexing the zygospores at maximum speed with glass beads for 5 minutes. The fixed cells were left in the refrigerator overnight at 4°C and then fixed and embedded as described earlier.

Sections were cut using a Dupont diamond knife and a Reichert Om u2 ultramicrotome and collected on 100 mesh, 150 mesh and 200 mesh grids for standard examination. Serials of approximately 100 sections were collected on open hole grids coated with formvar produced from a 0.3% (w/v) formvar in chloroform solution.

Collection of serial sections was carried out using a method similar to that developed by Rostgaard (1973). This method involves the cutting of a ribbon of approximately 100 sections, all light gold (approximately 800 Å in thickness). The sections were then guided to a forceps-held formvar coat grid using an eyelash mounted on a tooth pick. The forceps were attached to a rack and pinion device. This enabled one to lower the grid at the appropriate angle below the surface of the water. The ribbons of sections were then manoeuvered using an eyelash until they struck the formvar. The grid with the adhering sections was then raised using the rack and pinion until the slot was nearly filled with sections. The free end of the ribbon was released using the eyelash. This operation was repeated until the complete ribbon was collected.

Both serial and standard ultra thin sections were then stained for 3-5 hours in aqueous uranyl acetate (3% w/v) followed by .5-4 minutes in 0.25% lead citrate. Most preparations were viewed in a Philips 200 electron microscope at 60 KV or 80 KV, although some were examined in Philips 300 or Philips 201 model electron microscopes. All photographs were taken on 35 mm Kodak fine grain positive FRP 426 film. Photographs for reconstructions from serial sections were taken at a magnification which enabled the complete nucleus to fit on a single frame comfortably. Photographs of all sections through a nucleus were collected. Nuclei from which complete serials were

obtained were then printed on 8"x10" paper at the highest magnification which would allow the sections of the nucleus with the largest diameter just to fit. Tracings, one for each photograph, were made on 8½"x10½" 3M Brand projection transparencies.

Reconstructions of pachytene nuclei were carried out by tracing individual bivalents onto individual transparencies with the section number included. The alignment of transparencies from one section to the next was carried out using markers which traverse from one section to the next. The length traversed by a particular bivalent from section to adjacent section was calculated using the Pythagorean Theorum ($c=\sqrt{a^2+b^2}$). Bivalent reconstruction and calculation of their lengths was carried out using techniques similar to those of Moens and Perkins (1969) and Gillies (1972, 1973). Following the completion of tracing, the final reconstructions were produced on K&E 100% rag paper. These reconstructions were enclosed by an outline of the nuclear envelope. The section chosen for this was a centrally located one.

The model of the metaphase nucleus was reconstructed by tracing the outline of the nucleus from every second section onto foam rubber which had been cut to a thickness equalling that of two sections at this magnification. The outlines were then arranged in order and aligned as well as possible using markers which traverse from one section to the next. This is similar to the method used by Moens and

Rapport (1971) in their study of meiotic nuclei of yeast.

CHAPTER III

OBSERVATIONS AND RESULTS

A. Cytology

Cytological stages were assigned by two general criteria. The first criterion is the assignment of stage by analogy to the cytological features which occur during meiosis in other organisms. The second criterion is consistency with the stage sequence observed in other organisms.

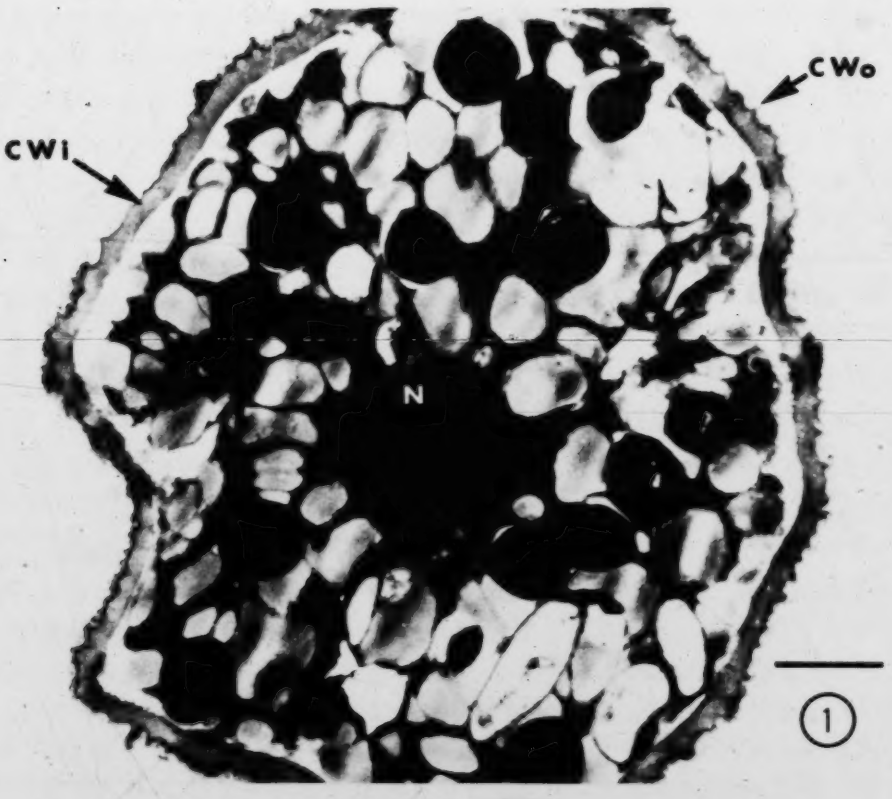
i) *Pre-meiotic stages*

The identification of pre-meiotic zygospores was based upon two cytological criteria, one of these being general morphology of the cells. Pre-meiotic zygospores could be subdivided into two morphological classes.

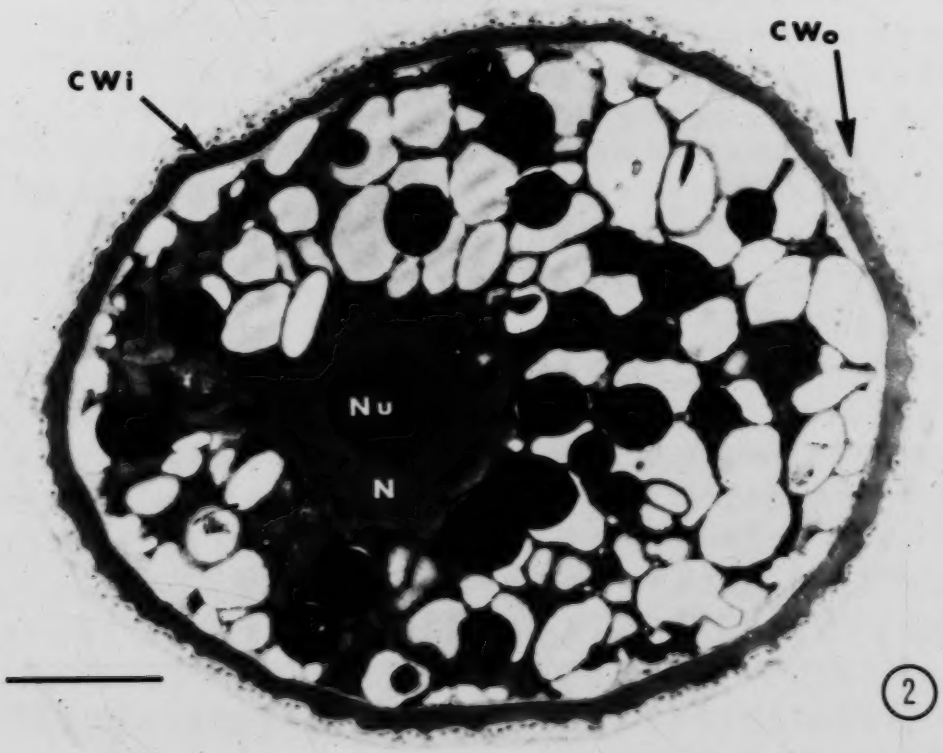
Early stage zygospores are similar to resting stage zygospores. The morphology of these cells as illustrated in Figure 1 is sufficiently distinctive to make them readily identifiable as early pre-meiotic. Both resting zygospores and zygospores up until four hours after the onset of germination are very dense. The cell walls are very thick, consisting of an inner electron dense region and an outer relatively electron transparent region. The inner and outer regions are each composed of several layers (Cavalier-Smith, 1967). Early in germination the cell is irregular in shape but steadily expands, until at

FIGURE 1. A resting stage zygote. Note the irregular outline prior to the onset of germination. Magnification 15,500. Bar = 1 μ

FIGURE 2. A cell typical of those observed at the pre-meiotic S period fixed at 5½ hours. A nucleolus (Nu) is present within the nucleus (N). Magnification 11,500. Bar = 2 μ



1



2

about 5 to 6 hours the cells are spherical. Figure 2 shows a typical cell at the completion of this initial phase of expansion.

The second cytological criterion was based upon nuclear morphology, this being the absence of the small patches of condensed chromatin with their associated less electron dense regions. This can best be illustrated by comparing the nuclei of Figures 1 and 2 with those of Figures 3, 5 and 6. The pre-meiotic nuclei are irregular, containing a nucleolus with a few scattered areas of condensed chromatin (Figure 2).

Both of the above stages (Figure 1 and Figure 2) can be shown to be pre-meiotic by a study of stage sequence. Thus, the fact that they show little cytological evidence of a change in nuclear morphology from the resting zygospore state, whereas a nuclear change is associated with zygospores at a later period following the onset of germination (leptotene, see below), identifies them as pre-meiotic.

In agreement with the observations of Cavalier-Smith (1974) no basal bodies were observed in cells up until the completion of this initial phase of expansion. This suggests that basal bodies are synthesized *de novo* at a later stage.

ii) *Leptotene*

The identification of leptotene zygospores was based

upon nuclear morphology alone. This was then verified by demonstrating that the morphological stage scored as leptotene occurred at a time consistent with the stage sequence observed in other organisms. Their nuclei clearly show small patches of condensed chromatin containing short discrete stretches of reduced electron density believed to be discontinuous lateral components.

Figures 3, 4, 5 and 6 show these and several other important aspects of leptotene cells. The nuclei are larger than those of zygospores at earlier stages and show a marked increase in condensation of chromatin over earlier stages.

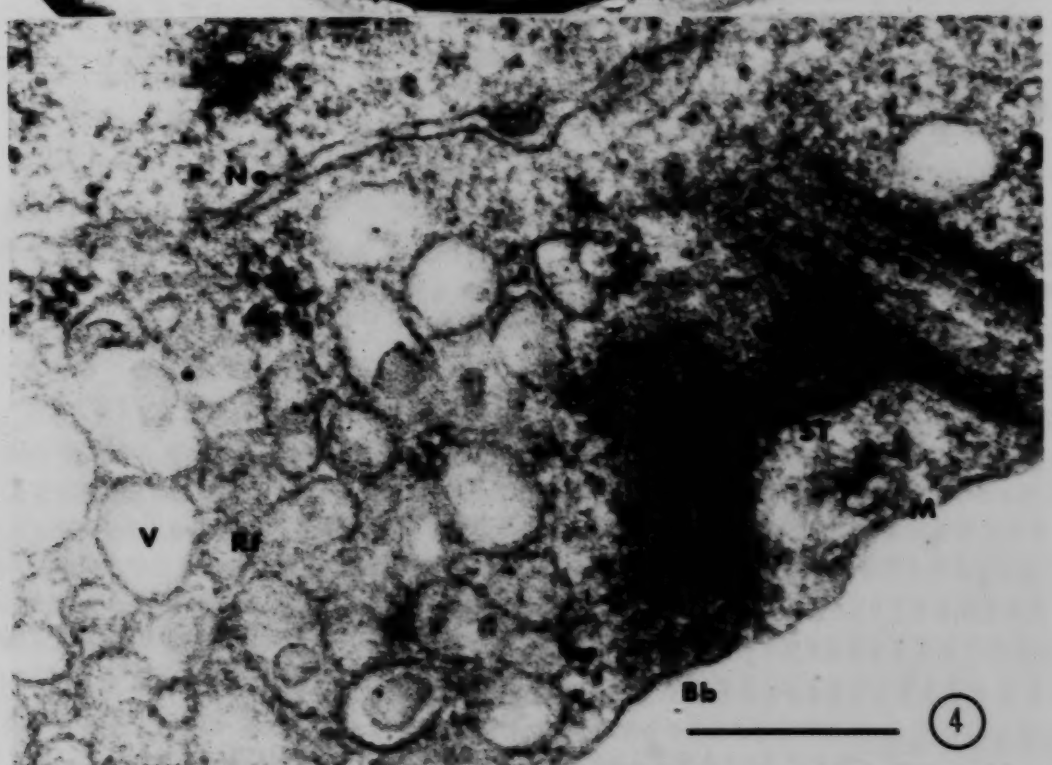
The condensed chromatin is found in small clumps scattered throughout the nucleus. The regions mentioned above, with electron density greater than that of the nucleoplasm yet less than that of the chromatin, are illustrated in Figures 3, 5 and 6. These regions are believed to be portions of lateral component which as yet are not continuous along the entire length of the chromosome. The leptotene nuclei of Figures 3, 5 and 6 all are less irregular than those of the pre-meiotic stages. The shape approximates that of a prolate spheroid with the surface nearest the interior of the cell somewhat collapsed. The nucleolus is located to one side of the nucleus and nucleolar organizer regions are evident.

Throughout early prophase the cells continue to expand, and by 6-7 hours most cells contain a space between

FIGURE 3. Early prophase cell fixed at 6.75 hours. Expansion has begun as evident by the space (S). The chromatin (arrows) is condensed into small patches. Adjacent to the nucleus is a basal body (Bb). The outer cell wall (CWo) is still intact. Magnification 18,000. Bar = 1 μ

FIGURE 4. Basal body (Bb) pair from an early prophase cell fixed at 6.75 hours. Lying between the basal bodies is a striated connection (St). Also present is an area containing vesicles (V) and free of ribosomes (Rf). Magnification 124,000. Bar = .25 μ

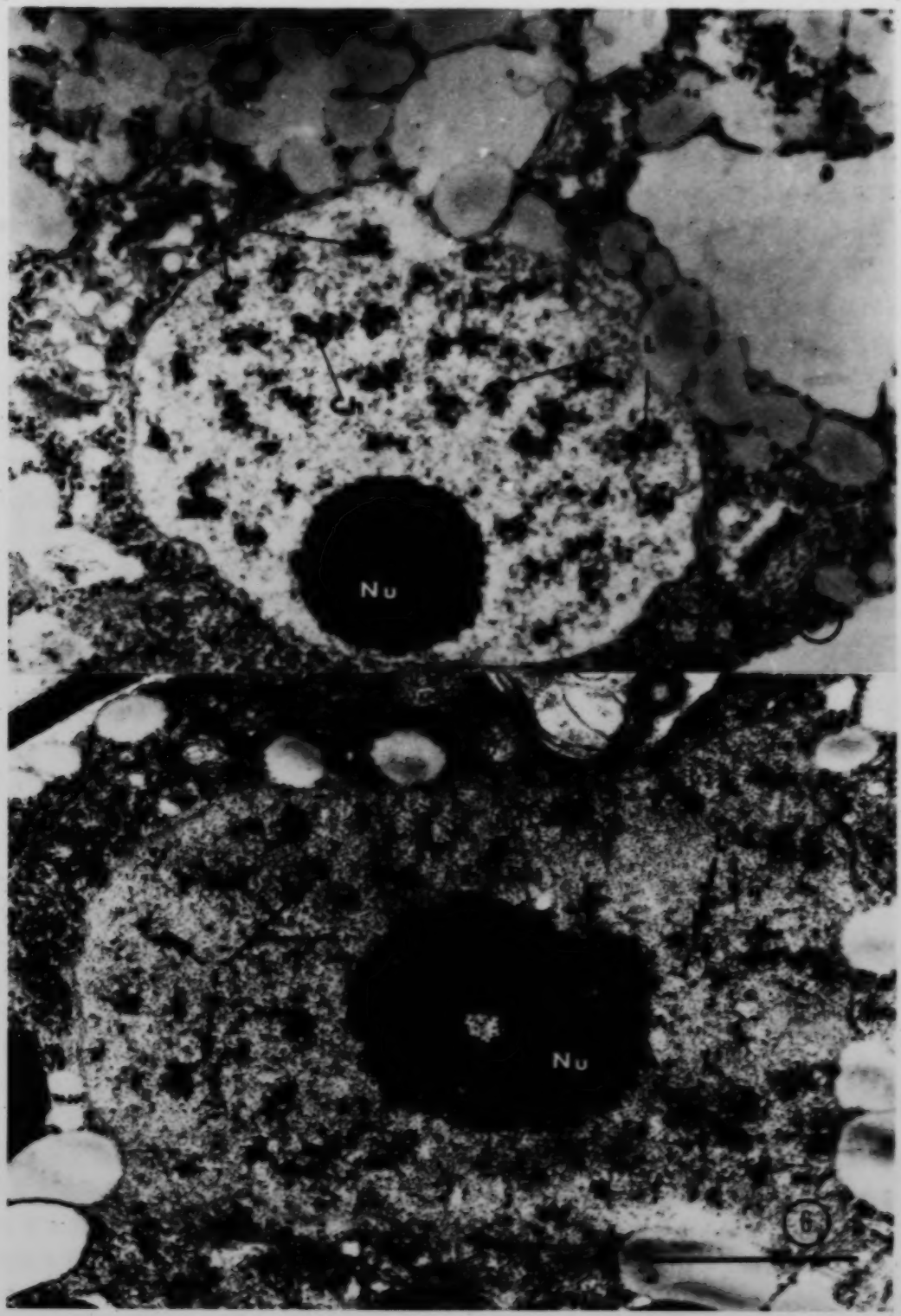
3



4

FIGURE 5. Leptotene nucleus from a spheroplast preparation. Scattered within the nucleus are small areas of condensed chromatin (Ch). Within the chromatin are found less electron dense areas believed to be stretches of discontinuous lateral components (L). Magnification 23,000. Bar = 1 μ

FIGURE 6. Leptotene nucleus of a cell with an intact cell wall. Notice the many small patches of chromatin. Some patches have less electron dense areas believed to be lateral components (L) associated with them. Magnification 36,000. Bar = 1 μ


Nu
Nu
6

the cell wall and the cell membrane (Figure 3). As the zygospores continue through prophase, expansion continues. Figure 7 shows a later stage zygospore with a very large space. Also evident in Figure 7 is the breakage of the outer layers of the cell wall and the stretching of the inner layers to accomodate the large increase in volume. The expansion of the cells and the accompanying breakage of the outer wall and stretching of the inner wall are similar to that reported by Cavalier-Smith (1967).

Figures 3 and 4 show that basal bodies are present adjacent to the nucleus during leptotene. Figure 4, a high magnification of a basal body pair in longitudinal section, shows that a mature pair is present in this leptotene cell. A connecting structure joins the two basal bodies, and a ribosome free area containing many vesicles surrounds the basal body pair.

iii) *Zygotene*

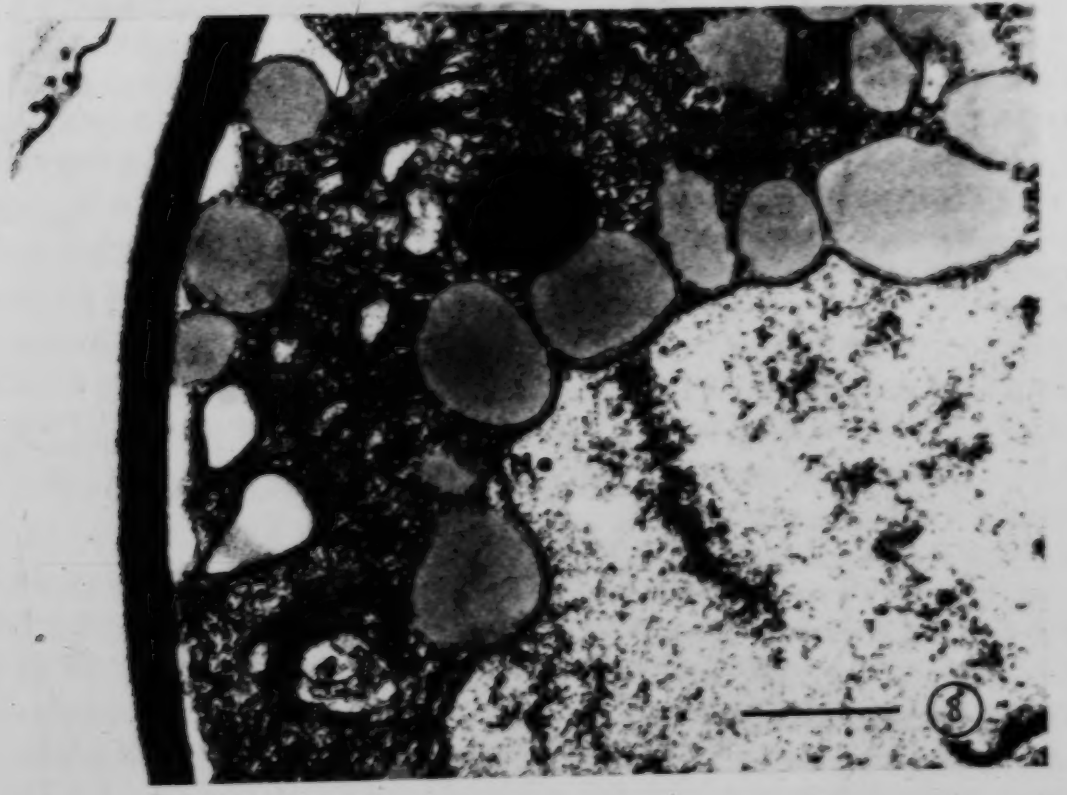
Zygospores were identified as being in the zygotene stage by the presence of long stretches of lateral component surrounded by chromatin, stretches of single chromosomes with lateral components, and stretches of lateral components from two chromosomes aligned at distances greater than 1,500 Å. Identification was then verified by demonstrating that the stage sequence was the same as that observed in other organisms.

Chromosome ends were regularly found to be attached

FIGURE 7. A prophase cell after expansion. Note the large space (S), the continuity of the inner cell wall (CWi) and the broken outer cell wall (CWo). Magnification 9,200. Bar = 2 μ

FIGURE 8. Portion of an early prophase nucleus showing a lateral component (L) and its attachment to the nuclear envelope (Ne). Magnification 47,000. Bar = .5 μ

⑦



⑧

to the inside of the nuclear envelope (Figure 8). The lateral component at this time appears to be a thin tubular structure approximately 800 Å - 1,000 Å in diameter (Figures 9, 10 and 11). Figure 9 shows a bivalent with a portion paired and a second portion as yet unpaired. The unpaired portion has the lateral component centrally located within a region of reduced electron density, 500 Å-600 Å across, which separates the chromatin. The lateral components of the bivalent shown in Figure 11 are separated by about 3,000 Å. It is evident from this micrograph that the chromatin is no longer found on both sides of the lateral component but is mainly on the side opposite the space between lateral components. When paired at about 1,200 Å, the lateral components are surrounded on three sides by chromatin (Figure 9). Figure 12 shows a zygotene nucleus with a stretch of lateral component within the nucleolus.

iv) *Pachytene*

Zygospores were classified as being in pachytene by nuclear morphology. Classification as pachytene required the presence of significant numbers of bivalent stretches paired at approximately 1,200 Å, with no pairing obviously greater than 1,200 Å. Figures 13-23, excluding Figure 18, show nuclei of zygospores which would be scored unequivocally as pachytene.

The prolate spheroid shape of the nucleus, observed

FIGURE 9. A portion of a zygotene nucleus. Present is a stretch of bivalent in the process of pairing. The portion to the right is paired at the appropriate distance (SC). The portion to the left shows a stretch where pairing is not completed and the lateral component (L) is found with chromatin (Ch) on both sides. Magnification 59,000. Bar = .5 μ

FIGURE 10. A portion of a zygotene nucleus showing a stretch of a homolog with the lateral component (L) running along the length of the stretch shown. The chromatin (Ch) is localized in chromomeres. Magnification 70,000. Bar = .3 μ

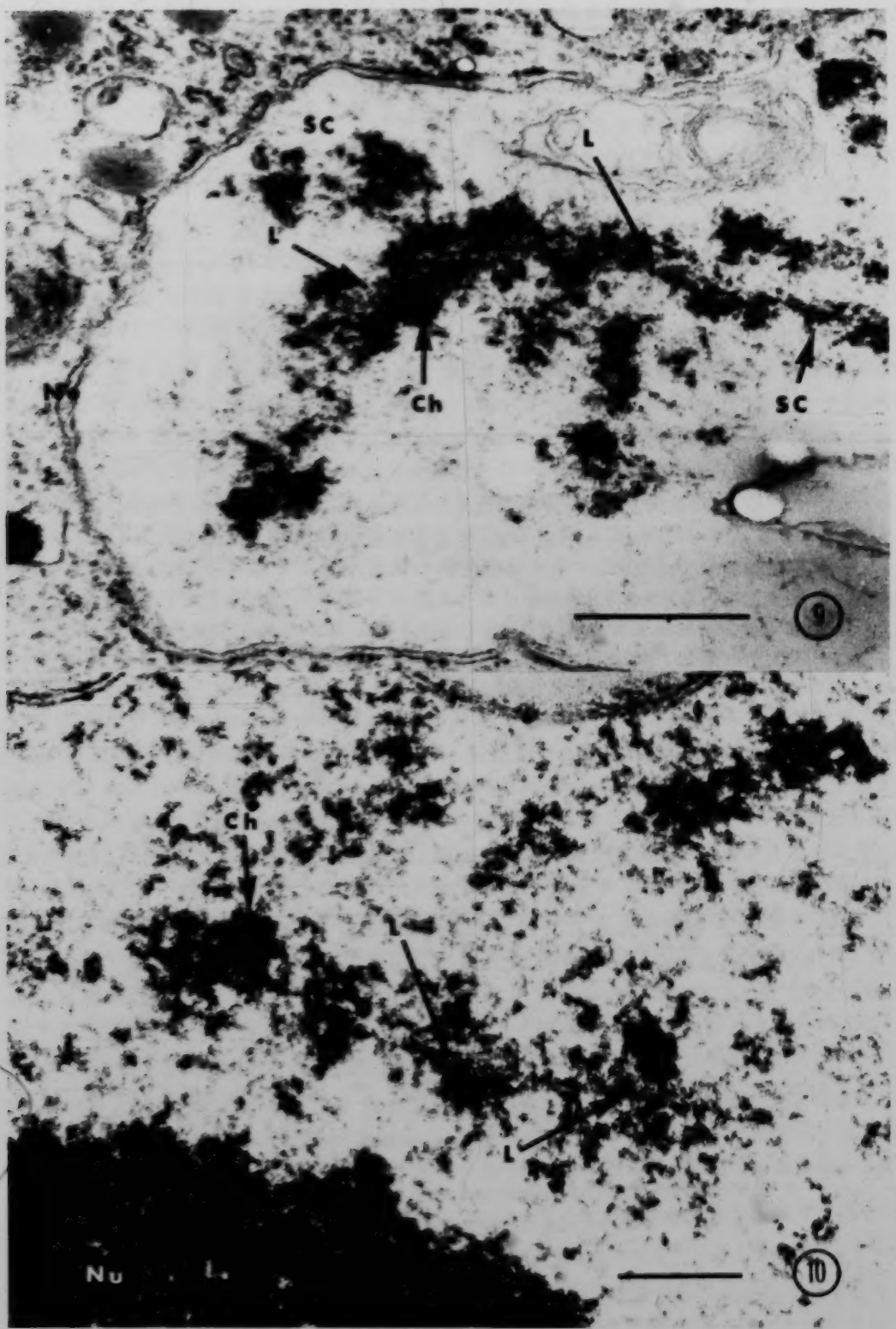

SC
L
L
Ch
SC
9
Ch
L
L
Nu
10

FIGURE 11. Portion of a zygotene nucleus. Present at about .3 μ apart are the two homologs (Ch) with their respective lateral components (L). As can be seen, the lateral components are still surrounded on both sides by chromatin. Magnification 52,000. Bar = .5 μ

FIGURE 12. A nucleus. Shown within the nucleus are several stretches of lateral component (L). The nucleolus (Nu) has a stretch of lateral component pass through it. Magnification 37,700. Bar = 1 μ

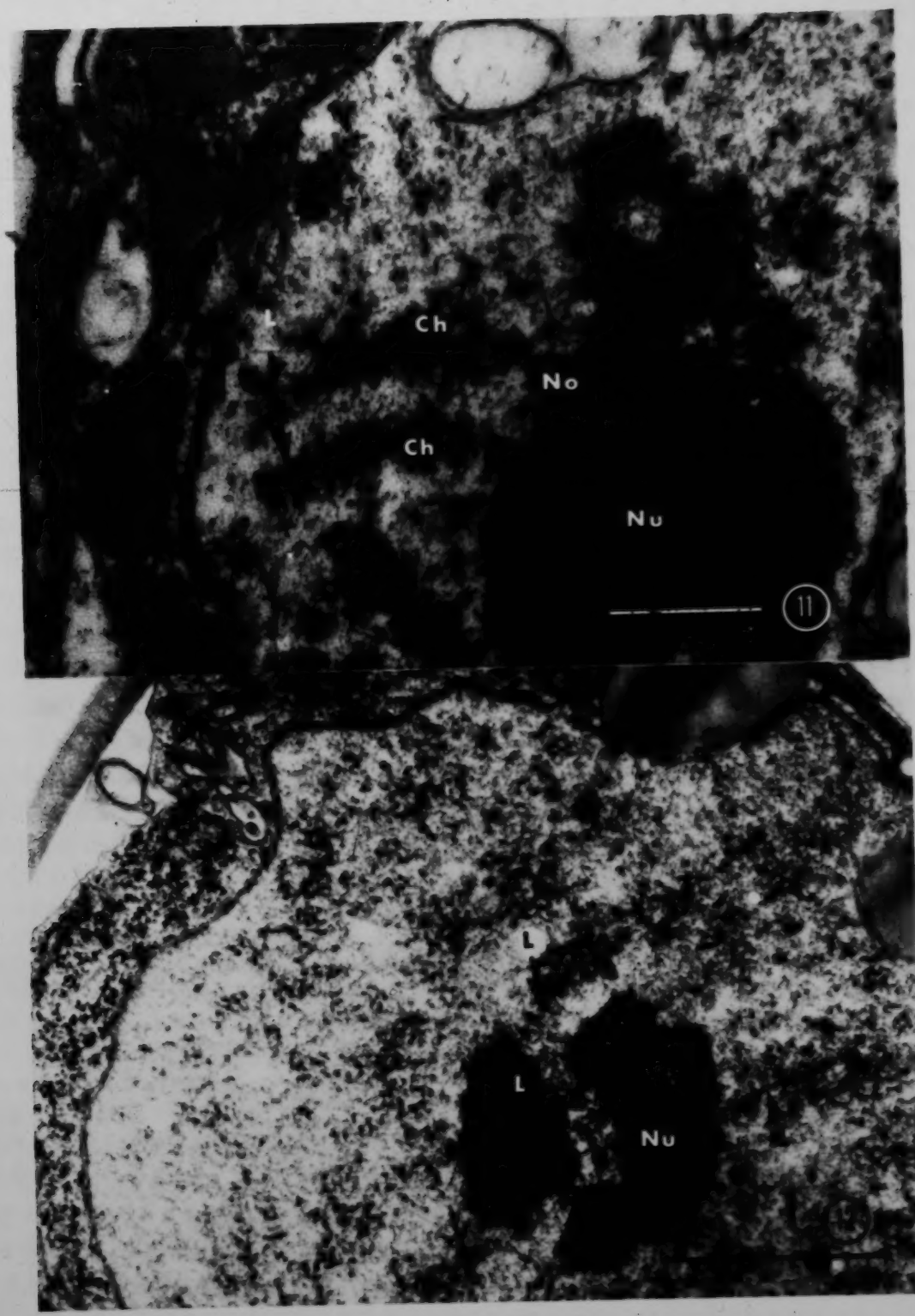

L
Ch
No
Ch
Nu
11
L
L
Nu

FIGURE 13. Pachytene cell fixed at 8½ hours. Present are remnants of the broken outer cell wall. The inner cell wall is still intact. In the upper portion of the cell is the space (S) similar to that shown in Figure 7. The nucleus (N) contains several stretches of synaptonemal complex (SC). Magnification 21,400. Bar = 1 μ

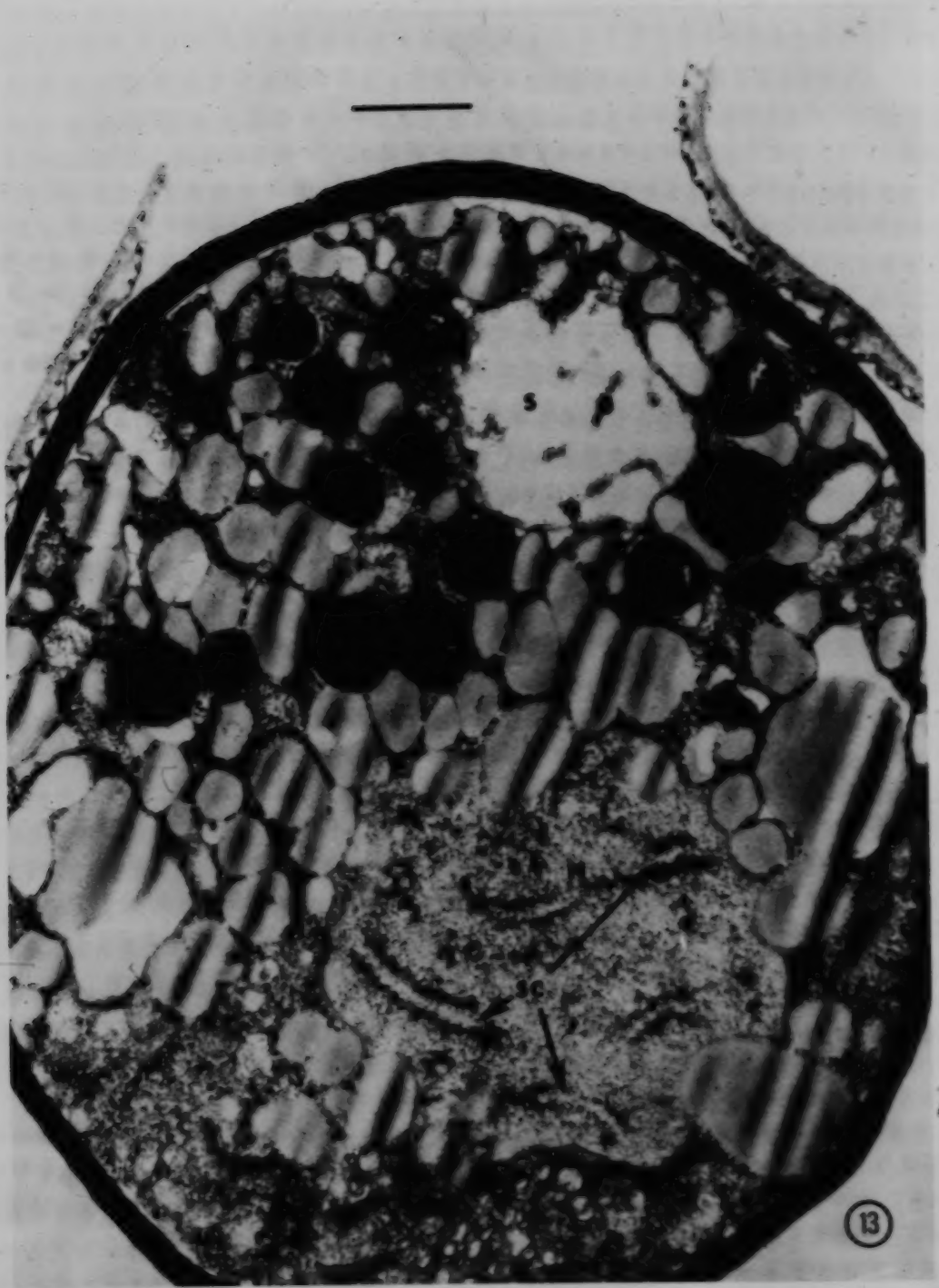
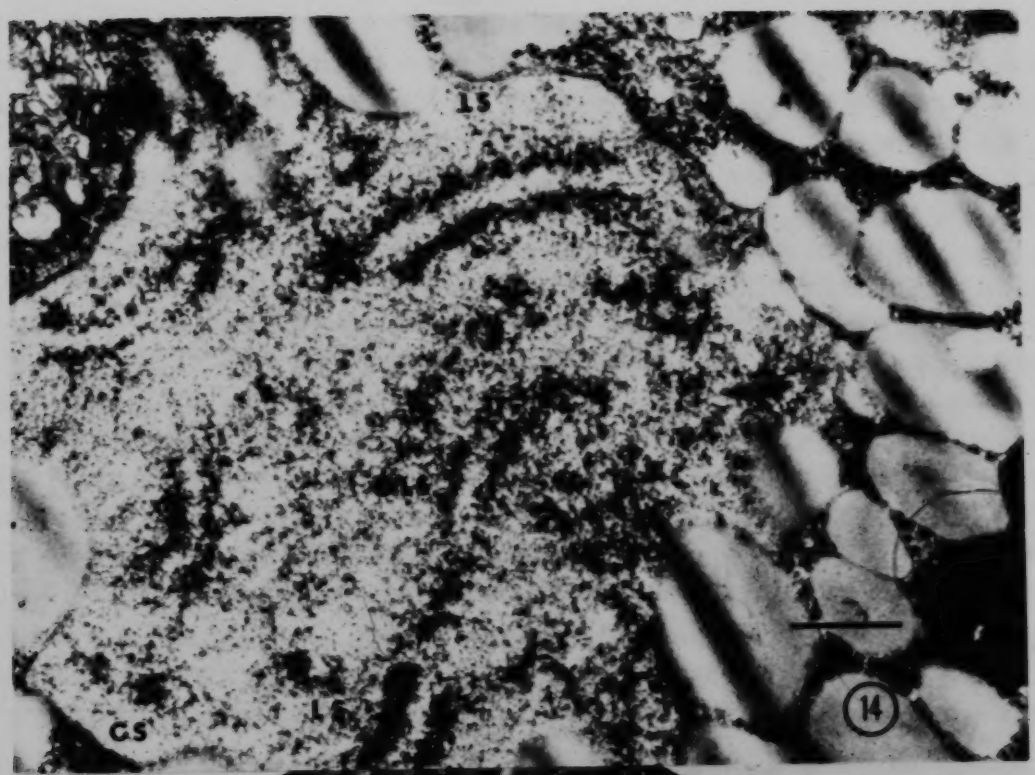

S
SC
13

FIGURE 14. A higher magnification of the nucleus shown in Figure 13. Included within the plane of the section are several lengths of synaptonemal complexes in longitudinal section (LS) and grazing longitudinal section (GLS) and cross section (CS). Magnification 34,100. Bar = .5 μ

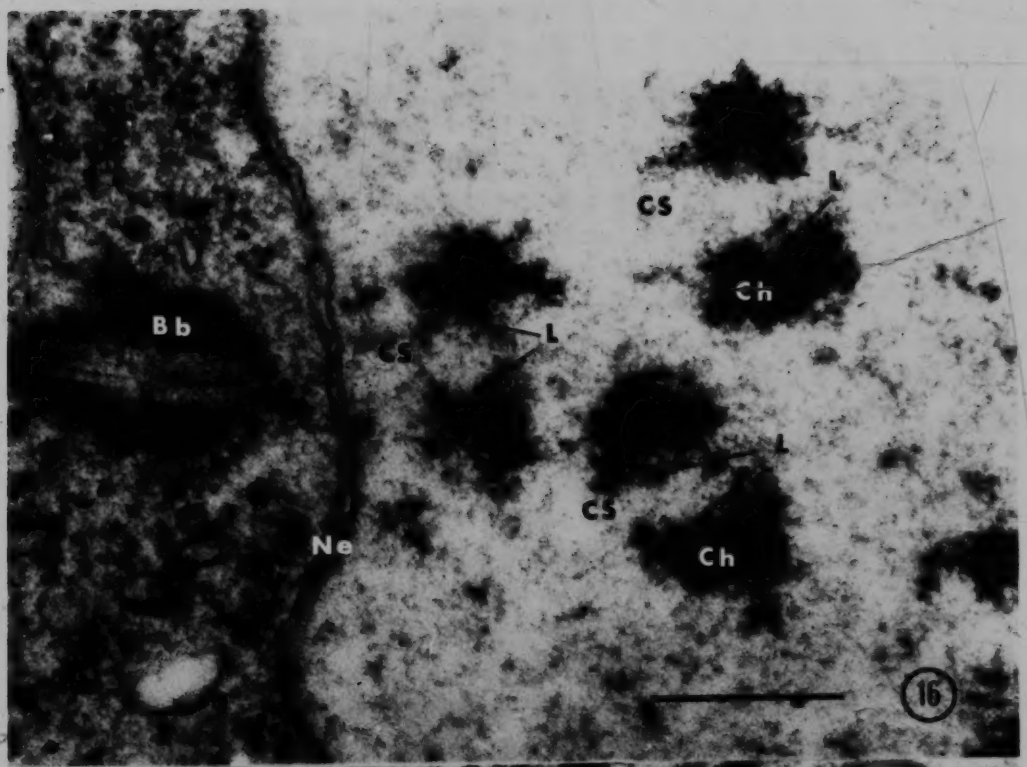
FIGURE 15. Pachytene cell fixed at 8.25 hours. Present within the cell are a space (S), a nucleus (N), a nucleolus (Nu), several synaptonemal complexes in cross section (CS) and a 4 μ length of synaptonemal complex. Magnification 13,900. Bar = 1 μ


CS
14

S
Nu
15

FIGURE 16. Synaptonemal complexes of *Chlamydomonas reinhardi* in cross section (CS). The chromatin (Ch) is distinguishable and forms a U shape around the lateral component (L). The nuclear envelope (Ne) separates the basal body (Bb) from the nucleus. Magnification 58,500. Bar = .5 μ

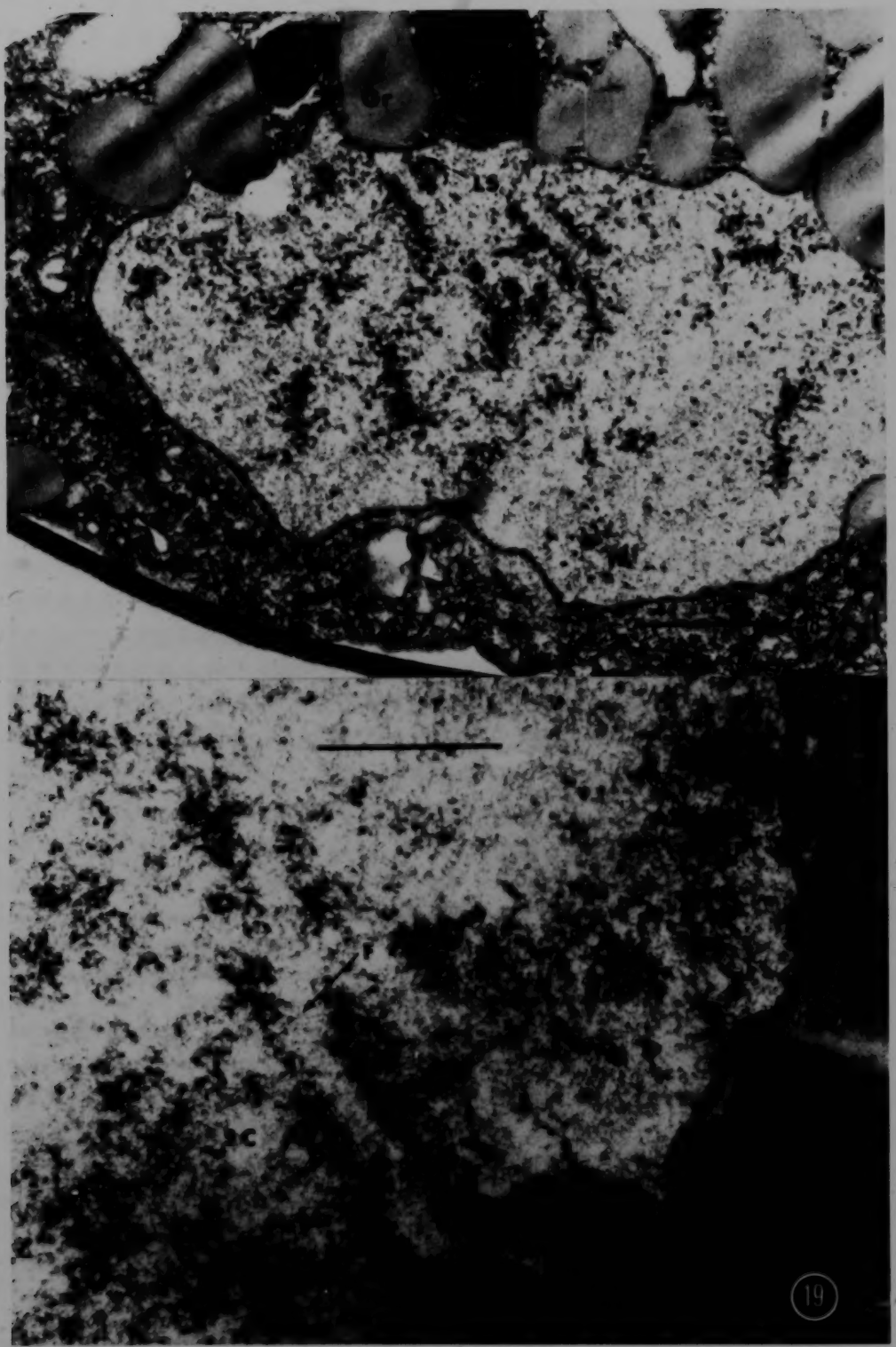
FIGURE 17. Synaptonemal complexes in grazing longitudinal section (GLS) and cross section (CS). Also present is a longitudinal section (LS) through one homolog of a bivalent pair showing the lateral component (L). Magnification 36,700. Bar = .5 μ


Bb
Ne
CS
L
CS
L
Ch
L
CS
Ch
16

LS
LS
GLS
Nu
CS
17

FIGURE 18. Section through a pachytene nucleus. One bivalent shown in longitudinal section (LS) is attached to the nuclear envelope opposite the portion of the nucleus nearest the cell wall adjacent to an area of granules (Gr). Magnification 21,700. Bar = 1 μ

FIGURE 19. Portion of a pachytene nucleus showing a synaptonemal complex (SC) attached to the nuclear envelope (arrow). Notice the filaments (F) joining the paired lateral components. Magnification 63,000. Bar = .5 μ


Cr
C
19

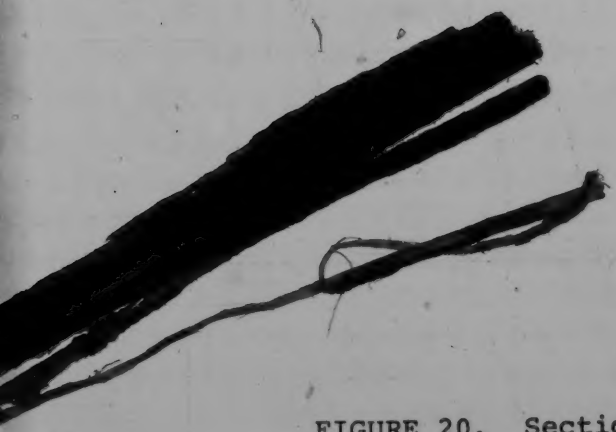


FIGURE 20. Section through a pachytene cell showing the nucleus. Contained within the nucleus are several bivalents in cross section (CS) and longitudinal section (LS). Present in the lower left portion of the nucleus is a dense mass of unpaired chromatin (DM). Present in the upper portion is a dense body (DB) similar to the recombination nodule described by Carpenter (1975a). Magnification 20,700. Bar = 1 μ

FIGURE 21. Portion of a pachytene nucleus showing a dense body (DB) and a mass of condensed chromatin (DM). Attached to the dense mass of chromatin, as evidenced by this section, are two bivalent arms (BA). Magnification 25,300. Bar = 1 μ

CS
CS
DB
LS
DM
CS
20
CS
Bi
Bi
BA
DM
Bi
BA
CS
21

in leptotene, is maintained through pachytene. This is demonstrated by the circular outline of the nucleus when sectioned in one plane (Figures 13, 15, 20 and 21), while Figure 23 demonstrates the elipsoidal aspect with one side pushed in, observed when sectioned at right angles to this plane.

Figures 13 and 15 show typical pachytene cells. Expansion is evidenced by the presence of a space and the breakage of the outer cell wall.

It was not possible to detect a central component between the lateral components of the paired bivalents in *Chlamydomonas* using methods of fixation which reveal a central component in most organisms, although the procedures used did fix and stain *Chlamydomonas* zygospores adequately with respect to other cellular components.

Figures 13 through 23 all show sections through pachytene nuclei. All these figures are of material fixed using method I and stained with 2% Ur acetate for 3-5 hours and .25% lead citrate for 2 minutes. All these figures demonstrate that *Chlamydomonas* does not show the characteristic complexes seen in most organisms during pachytene (see reviews by Moses, 1968; Sotelo, 1969; Westergaard and von Wettstein, 1972; Gillies, 1975a) when fixed and stained by this method. Rather the synaptonemal complex equivalent consists of two lateral components about 100 Å in diameter (Figures 16 and 17) which are slightly more

FIGURE 22. Portion of a pachytene nucleus showing a grazing longitudinal section (GLS) through a bivalent containing a dense node (D) in the central region. Magnification 64,600. Bar = .5 μ

FIGURE 23. Pachytene cell fixed at 7.75 hours. Several bivalents are shown in cross section (CS). Also present is a nucleolus (Nu) with 4 separate nucleolar organizer regions (NO). Magnification 26,000. Bar = 1 μ

GLS
N
Nu
LS
Nu
CS
CS
CS
Mo

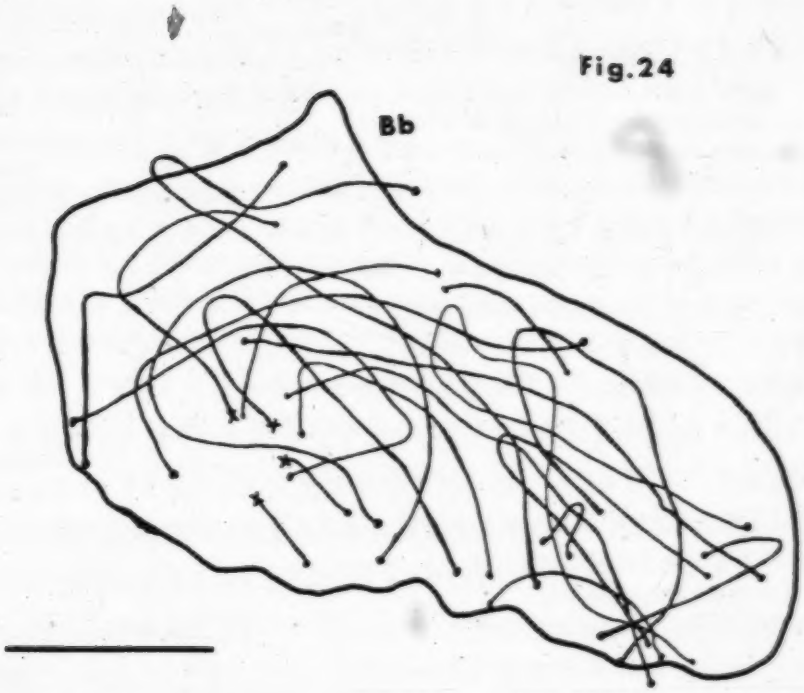
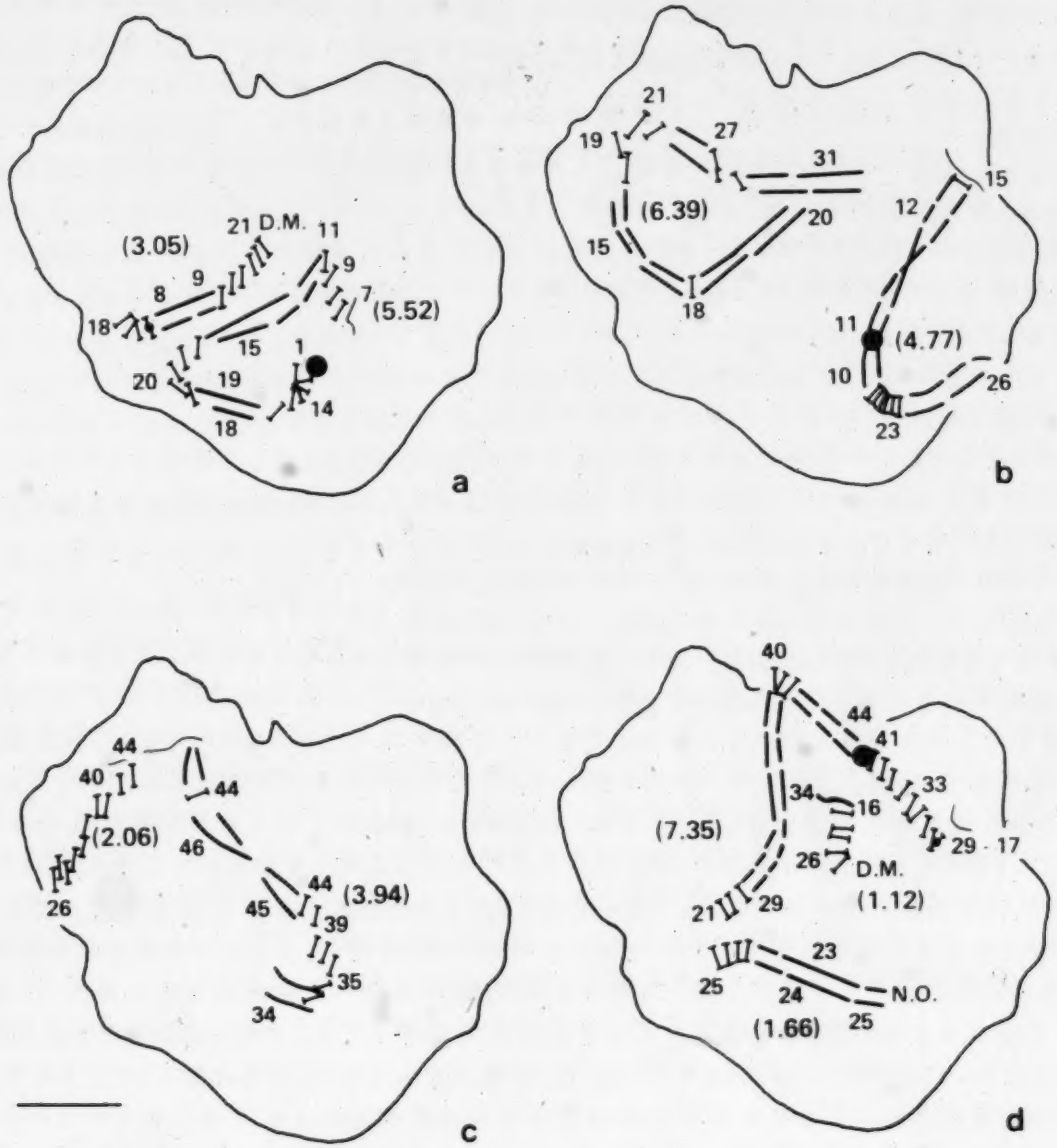


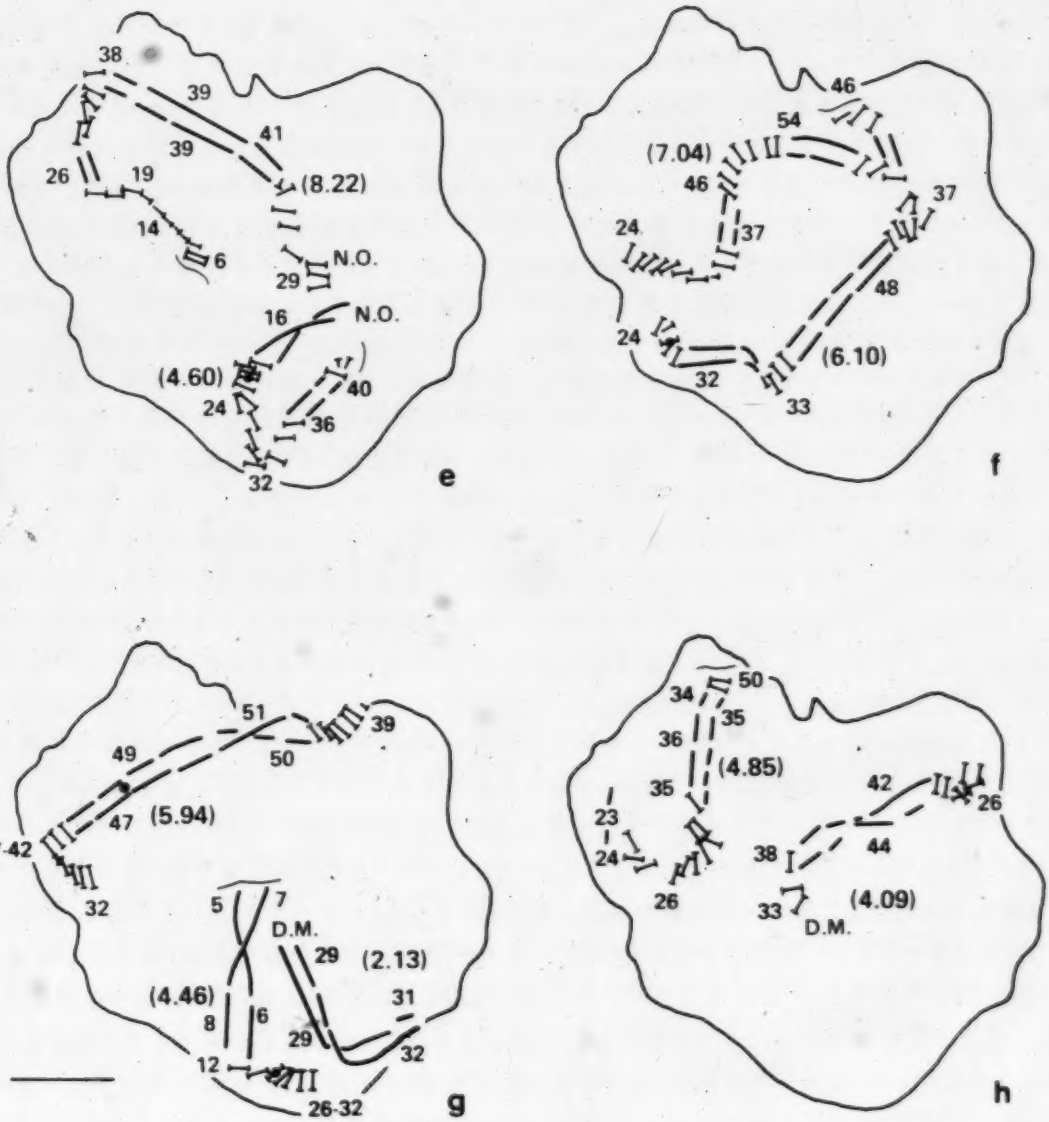
FIGURE 24. Reconstruction of nucleus # I showing the locations of the chromosomes placed on a centrally located section. Also indicated is the location of the basal body pair. Bar = 1 μ

Fig.25

FIGURE 25. Two-dimensional tracing of the reconstructed bivalents of nucleus #II. The ends of bivalent arms attached to the dense mass of presumed chromatin are marked by an x. Bar = 1 μ

Fig.26


(3.05)
21 D.M.
11
9
8
9
18
7
(5.52)
15
1
20
19
14
18
a
21
19
27
1
31
(6.39)
20
12
15
15
18
11
(4.77)
10
26
23
b
44
40
44
(2.06)
46
26
44
(3.94)
45
39
35
34
c
40
44
41
34
16
33
(7.35)
29
17
26
D.M.
21
29
(1.12)
23
25
24
N.O.
(1.66)
25
d


38
39
39
41
26
19
(8.22)
14
6
29
N.O.
16
N.O.
(4.60)
24
40
36
32
e
46
54
(7.04)
46
37
24
37
48
24
(6.10)
32
33
f
51
39
49
50
37-42
47
(5.94)
32
5
7
D.M.
29
(2.13)
(4.46)
8
6
31
29
32
12
26-32
g
50
34
35
36
(4.85)
35
42
26
23
24
38
44
26
33
(4.09)
D.M.
h

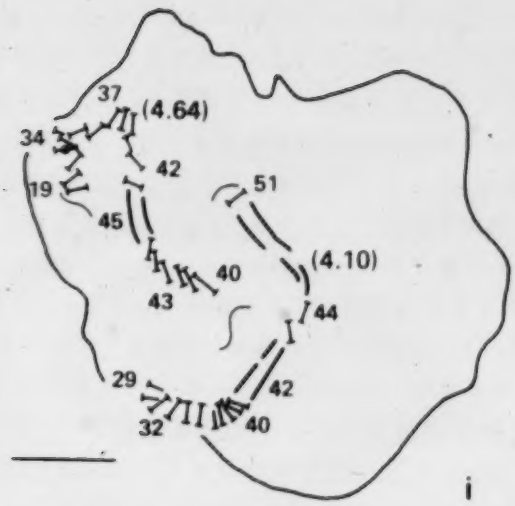


FIGURE 26 (a-i)

Reconstruction of bivalents and bivalent arms of nucleus #III. Numbers without brackets represent the section number in which portions of the bivalents occur. Numbers within parentheses identify bivalents by their arm length in μ. Small dots within bivalents indicate positions of dense nodes. The large circular spots represent the dense spherical bodies. DM indicates attachment to the dense mass of presumed chromatin; No, sites of nucleolar organizer regions. Thin lines present near the ends of bivalents which approach the nuclear membrane indicate the position of ends relative to the nuclear membrane. Bar = 1 μ

TABLE I. Lengths of bivalents from three reconstructed nuclei of *Chlamydomonas reinhardi* and the distance of nucleolar organizers (a), "nodes" (b), and "recombination nodules" (c or d) from one end of the appropriate bivalent. Bivalents ending in the dense mass shown in Fig. 20 and 21 are designated by (e). Lengths of bivalents in nucleus III are given to two decimal places only to enable each bivalent to be identified by length.

Nucleus I		Nucleus II		Nucleus III	
Length (µm)	Distance from one end of bivalent (µm)	Length (µm)	Distance from one end of bivalent (µm)	Length (µm)	Distance from one end of bivalent (µm)
8.2		6.4	3.1[cd]	8.22	0.27[a]
7.2		6.3		7.35	0.55[b]
					2.55[d]
6.6	2.3[a]	5.5		7.04	
6.1		5.5	1.7[b]	6.39	
5.8		5.3	1.6[b]	6.10	0.28[b]
4.8		5.1	1.0[a]	5.94	2.2[b]
4.5		5.1	2.4[c]	5.52	0.8[c]
4.4		5.1	0.9[d]	4.85	
4.4		4.0		4.77	2.2[c]
4.2	0.1[a]	3.9		4.64	1.3[b]
	0.6[c]				
4.1		3.3	0[a]	4.6	1.5[b]
					0[a]
4.1	0.6[a]	3.2	0[a]	4.46	2.4[b]
			2.6[b]		
3.6	1.6[b]	3.0		4.10	
	3.0[c]				
3.2	1.0[d]	2.7		3.94	
2.4		2.5		2.06	
1.8	0.2[a]	2.3		1.66	0[a]
3.0[e]	0.9[b]	2.1[e]		4.09[e]	
2.0[e]		1.9[e]		3.05[e]	1.0[b]
0.6[e]		1.0[e]		2.13[e]	
0.5[e]		0.6[e]		1.12[e]	

TABLE I.

electron dense than the nucleoplasm but considerably less dense than the chromatin associated with them. These two lateral components are separated by a distance of about 1,200 Å (Figure 16). Running across the central region are filaments of about 60 Å in diameter (Figure 19); these filaments join the two lateral components. The chromatin is condensed around each individual lateral component in a U shape and is usually separated from the chromatin of the other lateral component of the pair. Lateral components are essentially amorphous at this time and run the entire length of most homologs (Figure 17) at the beginning of pachytene. Early in pachytene the lateral components of some of the bivalents terminate on the nuclear membrane (Figures 18 and 19), but as pachytene proceeds the nuclear membrane associations are lost, although most of the ends do remain close to the nuclear envelope.

As pachytene proceeds the lateral component becomes more difficult to observe although the continuity of pairing is maintained. This continuity is best demonstrated in Figure 15 where a length of bivalent 5 μ long is found in the plane of the section and in the reconstructions of Nuclei I, II and III shown in Figures 24, 25 and 26.

Using a different procedure for the fixation of zygospores (method II) the bivalent pairing described above turned out to be similar to that seen in other organisms. The method involved the removal of the cell wall by

vortexing with glass beads 10 minutes after the initial fixation with glutaraldehyde was initiated. Figures 27a and 27b show cells with the cell wall removed. The nucleus has remained intact and the cytoplasm and chloroplast have remained intact as well. Unfortunately only a small portion of the cells treated in this fashion have the cell wall removed and yet are still in a fairly intact condition for analysis by electron microscopy. An interesting feature of the wall-less zygospores produced by this procedure is the reduced electron scattering ability of the nucleoplasm. This is probably due to leaching. It may be that this leaching of material from the nucleoplasm enables the detection of a central component (Figures 28 a-d).

As in the central region of the cells fixed by method I (*e.g.* Figure 19), method II reveals filaments of material 50 Å in diameter which run between the lateral components of the two homologs. The central component is clearly distinct from these. It is about 60 Å wide, and runs down the middle of the central region in a somewhat irregular zig zag path with deviations showing an amplitude of 150 Å-200 Å and a periodicity of 500 Å (Figures 28a, 28b and 28c).

Because of the continuity of pairing during pachytene, it was possible to carry out pachytene reconstructions of three nuclei from serial sections. Nucleus I was reconstructed from 65 sections, nucleus II from 47 sections

FIGURE 27 A & B

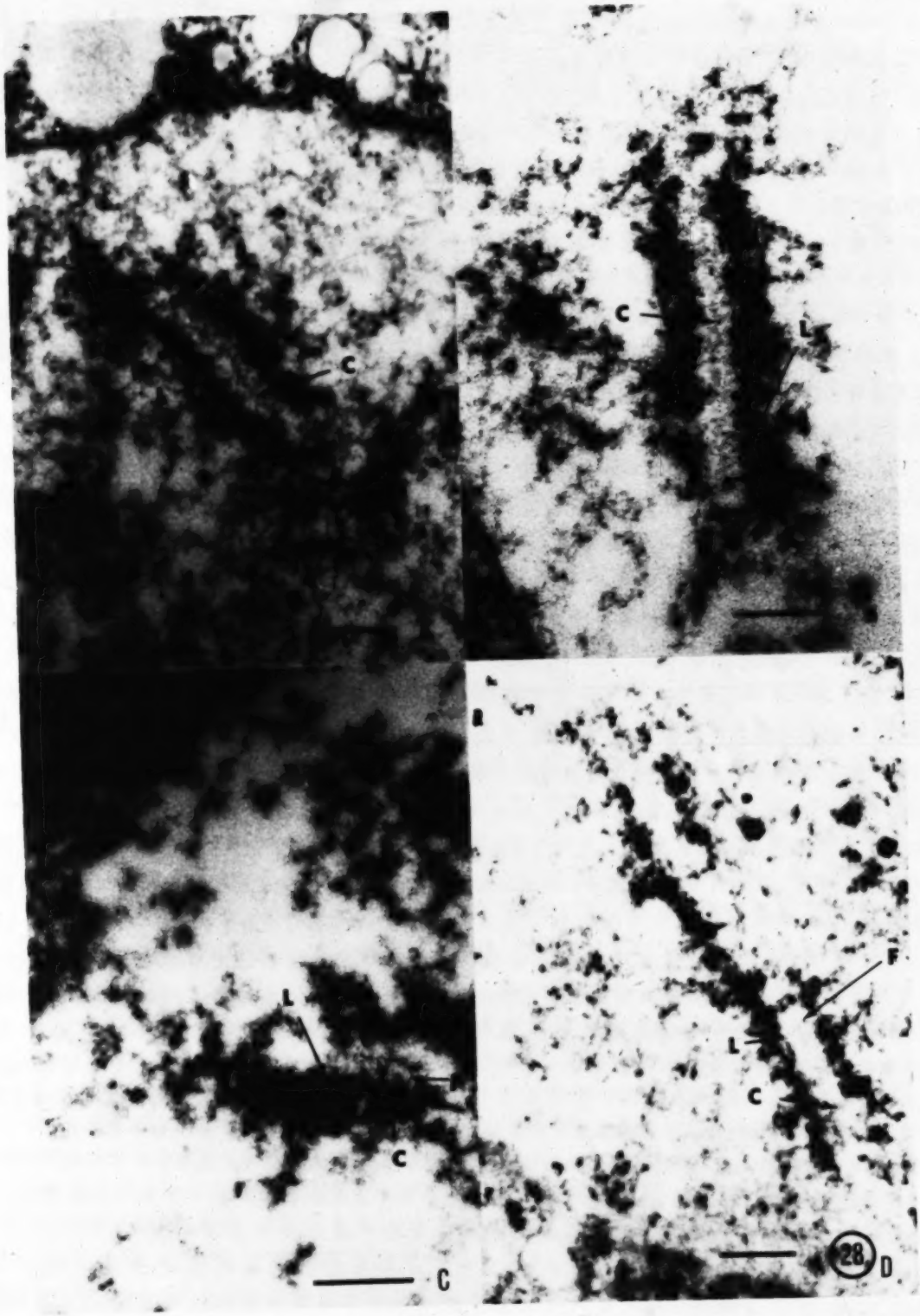
Pachytene spheroplasts (Sp) prepared by vortexing with glass beads. Note the intact nature of the cells and the lack of a cell wall. Also present in Figure A are two empty cell walls (CW) and an intact cell.

Magnification A 6,750 Bar = 2 μ
B 6,750

CW
Nu
Sp
27 A
Nu
Sp
27 B

FIGURE 28 a, b, c & d
Bivalents from 4 different pachytene cells which lack a cell wall. Prepared by Method II, vortexing with glass beads. Note the central element (C) in all four examples.

Magnification a 50,000 Bar = .25 μ
b 61,000
c 70,000
d 50,000


C
C
L
L
C
C
F
L
C
28
D

and nucleus III from 61 sections.

It was possible to follow all bivalents along their entire paired length, thus identifying 16 individual bivalents and four other bivalent arms attached to a mass of chromatin. This mass of chromatin did not show pairing nor did it show continuous lateral elements running through it (Figure 21).

Assuming the four arms could represent a maximum of four individual bivalents and a minimum of two individual bivalents, it can be concluded that *Chlamydomonas reinhardi* has from 18-20 bivalents. The total length of paired chromosomes was 81.5 μ in nucleus I, 75.1 μ in nucleus II, and 92.0 μ in nucleus III. Figures 24, 25 and 26 show reconstructions of nuclei I, II and III respectively. Table I shows individual bivalent lengths and positions of interesting features in all three nuclei.

It was possible to distinguish four individual nucleolar organizer regions (Figure 23) associated with the nucleolus of two nuclei and five nucleolar organizer regions in the other nucleus, but only three nucleolar organizer regions in any nucleus had synaptonemal complexes associated with them. Only the position of definite attachments are listed in Table I. It is to be noted that only three bivalents show attachment to the nucleolus in any one nucleus. Each nucleus has a different complement of bivalents showing attachment to the nucleolus.

Most chromosomes were found to approach within 0.2 μ

of the nuclear envelope at both ends. Those ends that approach the nuclear membrane and those that do not are indicated for nucleus III in Figure 26.

Nodes similar to those observed by Gillies (1972), Zickler (1973) and Byers and Goetsch(1975) were regularly found in pachytene nuclei. These nodes were found to be fairly uniform in size. Their dimensions were about 500 Å x 800 Å in longitudinal section and 500 Å x 500 Å in cross section. Their positions,as observed in nucleus III are shown in the reconstruction in Figure 26 and their distances from the chromosome ends for all three nuclei reconstructed from serials are given in Table I.

Another interesting feature of pachytene nuclei is the presence of spherical bodies ranging from 1,200 Å-2,500 Å in diameter (Figures 20 and 21). These bodies are more electron dense than the chromatin and are associated with the bivalents. Two of them were found in each of the three nuclei examined completely by serial sections. These electron dense bodies are similar in size and structure to those reported by Carpenter (1975a). Two observations from *Chlamydomonas* that are significantly different from those made by Carpenter should be described because they relate directly to her hypothesized role for them. The first is that one spherical body has been observed in association with two bivalents (Figure 21). The second is that two spherical bodies have been seen associated with one bivalent, the distance between them being .2 μ.

The reconstruction of nucleus I shown in Figure 24 clearly reveals the presence of a bouquet arrangement during pachytene in *Chlamydomonas*. On the other hand, careful analysis of Figure 26 a-i, the reconstruction of nucleus III, indicates that the bivalent ends are not all found in one area. This may indicate that nucleus III is further into pachytene and the bouquet arrangement has started to break down. Hence it may be concluded that the bouquet arrangement is a transient one, appearing first at the beginning and disappearing before the end of pachytene. It should also be noted that there is a single basal body pair on the opposite side of the nucleus to where the chromosomes attach in Figure 24. There was also a single basal body pair associated with nuclei II and III.

In addition, in nuclei II and III,it can be seen that, although the bouquet arrangement has broken down, the basal body pair still remains to the side of the nucleus opposite the area where most bivalent ends are found. Examination of the micrographs in Figures 8, 18 and 19 and the reconstruction of nucleus I (Figure 24) shows that the bivalent ends are attached to the face of the nucleus adjacent to a group of electron dense granules.

Basal bodies at pachytene are shown in Figures 29, 30, 31 and 32. Figure 29 shows a basal body pair in longitudinal section. Both members of the pair are approximately .25 μ in length and lie with a 45° angle between their major axes. Figure 30 shows a high magnification of

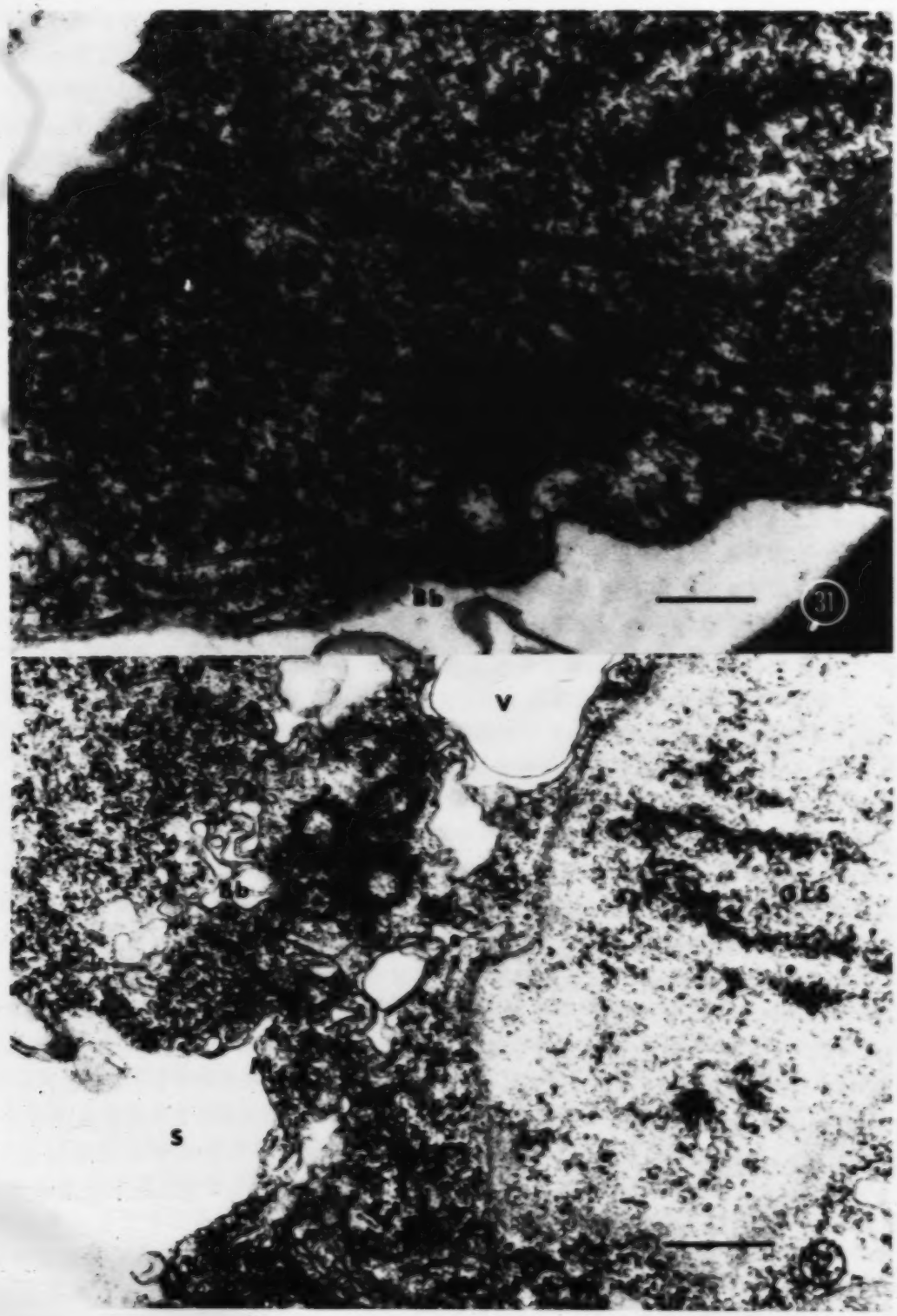
FIGURE 29. Longitudinal section through a basal body (Bb) pair from a pachytene cell. Notice the ribosome free area (Rf) and the associated vesicles (V). Magnification 88,200. Bar = .25 μ

FIGURE 30. Cross section through a basal body (Bb). Note the triplet structure of each of the nine units of the structure. Magnification 160,000. Bar = .2 μ

Ne
Rf
Bb
V
29
30

FIGURE 31. Pachytene cell fixed at 7.75 hours. Shows a basal body (Bb) lying between the nuclear envelope (Ne) and the cell membrane (M). Microtubules (Mt) radiate out from the basal body. Magnification 68,000. Bar = .25 μ

FIGURE 32. Group of four basal bodies (Bb) adjacent to the nucleus (N) and the cell membrane (M) of a pachytene zygospore. There is also a large vesicle (V) adjacent to the basal body pair. Magnification 38,200. Bar = .5 μ


Bb
31
V
Bb
OLS
S

a basal body in cross section in which the typical cart-wheel structure is evident. Figure 31 shows extranuclear microtubules associated with a basal body pair at pachytene. Figure 32 shows a group of 4 basal bodies adjacent to a pachytene nucleus; this was observed rarely. It should be noted that, of 7 pachytene nuclei examined by serial sectioning, all were found to have a single basal body pair associated with them.

v) *Diffuse stage (Diffuse diplotene)*

The classification of zygospores as diffuse stage was based upon nuclear morphology. Nuclei containing no obvious condensed chromatin, very diffuse chromatin, or diffuse chromatin paired at distances greater than 1,200 Å, were scored as diplotene. This classification was then verified by its stage sequence.

Figures 33 and 34 are both micrographs of cells scored as diplotene. Several areas of diffuse chromatin are present in both figures. In the upper right hand portion of the nucleus in Figure 34 lies a 1.5-2 μ stretch of bivalent, which illustrates the diffuse nature of the chromatin at this time. Two sites approximately 1 μ apart are indicated as possible chiasmata present in this bivalent; at least, these structures and ones similar to them are the most obvious candidates for chiasmata.

Nucleoli are still present within nuclei at this stage (Figure 34).

FIGURE 33. Diffuse diplotene cell fixed at 8¼ hours. Notice the general decondensed nature of the chromatin within the nucleus (N). Magnification 17,000. Bar = 1 μ

FIGURE 34. Another diffuse diplotene cell fixed at 8¼ hours. Present within the plane of the section is a nucleolus (Nu) and a diffuse bivalent (Bi) with possible chiasmata (Chi) in the upper right hand side of the nucleus. Magnification 22,000. Bar = 1 μ

33
Ch
Chl
Ch
Ch
Nu
Nu
S
34

vi) *Late prophase I (Diakinesis)*

Zygospores containing nuclei with large areas of irregular condensed chromatin scattered throughout were classified as late prophase I.

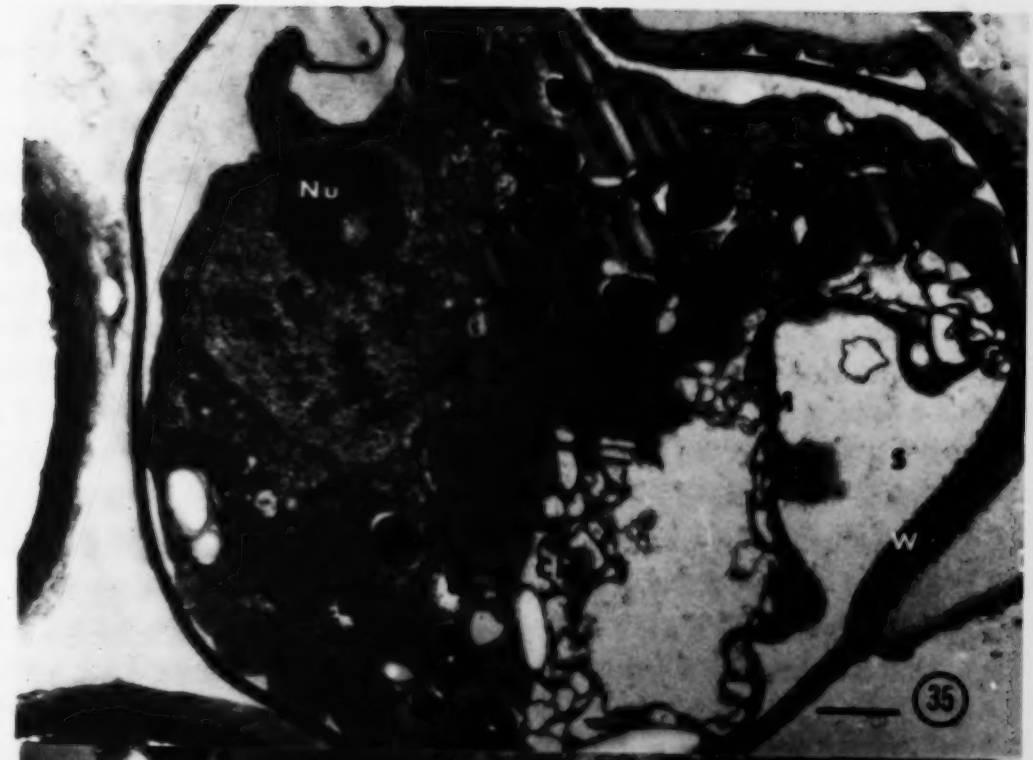
Figures 35, 36 and 37 are from zygospores scored as late prophase I. All three show extensive areas of irregularly shaped condensed chromatin scattered throughout the nucleus. The nucleoli in Figures 36 and 37 are irregular with extensive electron transparent invaginations. These nucleoli are believed to be undergoing nucleolar breakdown, in contrast to the spherical nucleoli seen at earlier (Figures 34 and 23) and later stages (Figures 51 and 57). Two basal body pairs removed some distance from one another but still in association with the nucleus are regularly seen at this time (Figure 37).

vii) *Metaphase*

Examination of metaphase nuclei from complete serial sections revealed several interesting features. Metaphase nuclei lack a nucleolus. As at prophase, each basal body pair has a ribosome free area which now radiates extranuclear and nuclear microtubules (Figure 38). Between the intranuclear spindle and the basal body region is a polar fenestra (Figure 38). The chromatin which began condensing during late prophase aggregates early in metaphase (Figure 39). A higher magnification shows microtubules scattered throughout the nucleus (Figure 40).

FIGURE 35. Late prophase cell fixed at 8½ hours. Irregular areas of condensed chromatin (Ch) are found scattered throughout the nucleus. A large space (S) is present between the cell membrane (M) and the zygote wall (W). Magnification 12,500. Bar = 1 μ

FIGURE 36. Higher magnification showing the nucleus of another late prophase cell. The nucleolus (Nu) has started to break down. Less electron dense invaginations (In) are present within the nucleolus. Magnification 33,900. Bar = .5 μ


Nu
W
35

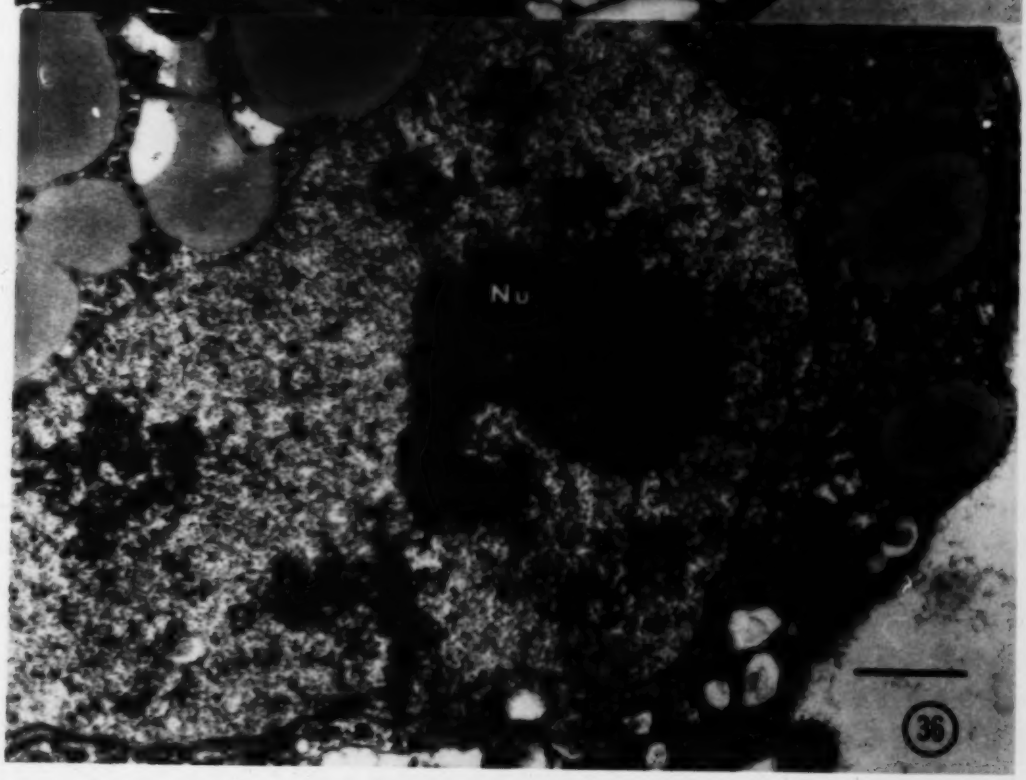

Nu
36

FIGURE 37. Nucleus of a cell in late prophase I. The nucleolus (Nu) has started to break down. Basal body pairs (Bb) are found separated some distance from one another next to the cell membrane (M) and the nuclear envelope (Ne). Magnification 32,600. Bar = 1 μ

FIGURE 38. Spindle pole of a metaphase I cell. The spindle pole is shown here with a single basal body (Bb) in the plane of the section. Microtubules (Mt) approach the basal body through a polar fenestra (Pf) in the nuclear envelope (Ne). Magnification 64,800. Bar = .25 μ

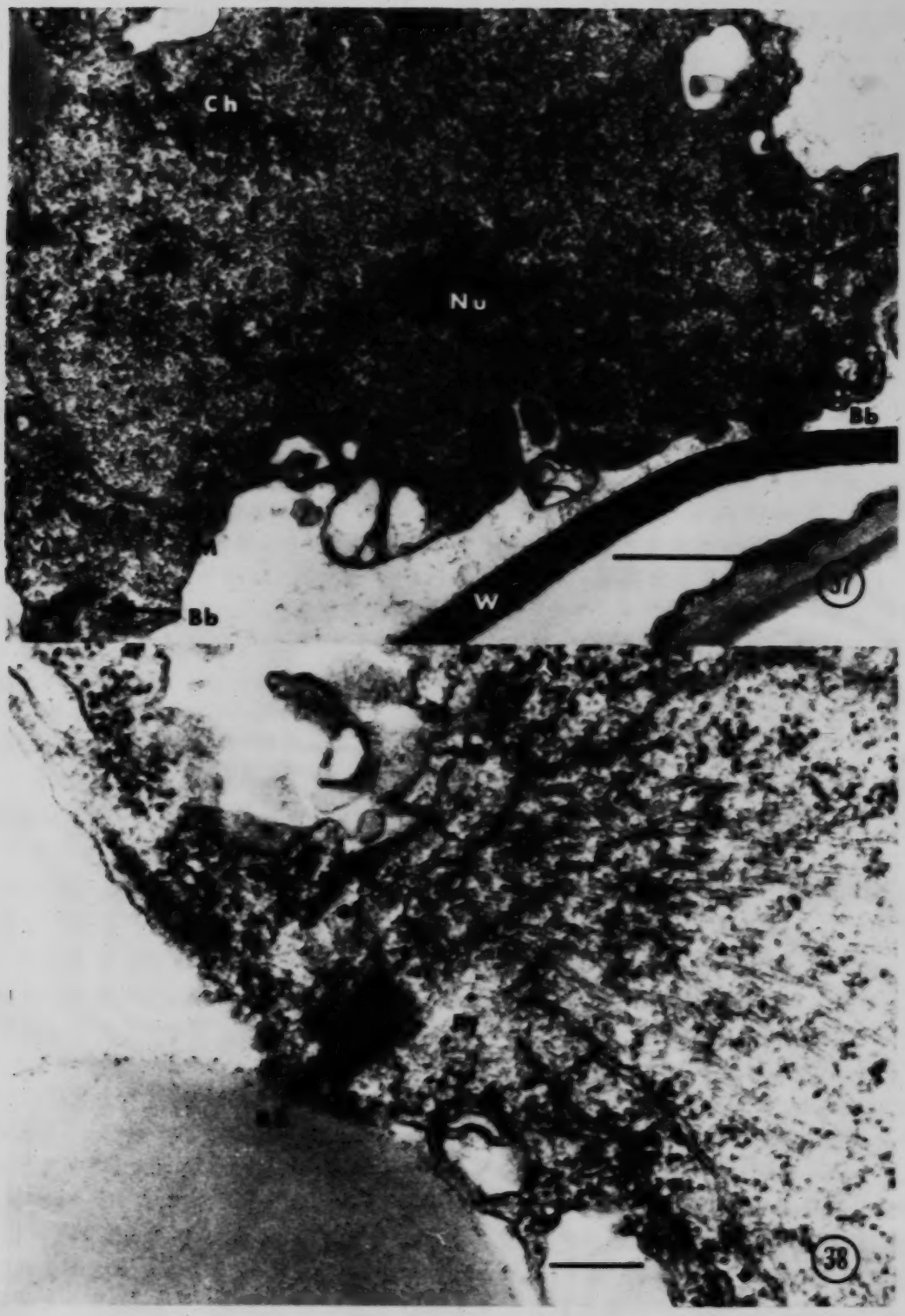

Ch
Nu
Bb
W
Bb
37
38

FIGURE 39. Early metaphase cell fixed at 8½ hours. The nuclear envelope (Ne) is somewhat irregular. The chromatin (Ch) is just beginning to aggregate. The chloroplast (Chl) still has not formed distinct stacked lamelae. Magnification 23,100. Bar = 1 μ

W
S
Ch
39

The reconstruction shown in Figure 41 is of an early- to mid- metaphase nucleus. This model together with appropriate figures demonstrate the following features of the metaphase I nucleus. Early in metaphase, the nuclear membrane possesses evaginations giving it an irregular shape. Later, as shown by Figure 43, the outline is smoother. There are two basal body pairs per zygospore at metaphase I, one pair being found at each pole. The nucleus is of a crescent shape, so that both poles extend in towards the cell's interior while maintaining their positions adjacent to the cell wall. Thus the actual distance between basal body pairs is 5.0 μ, while the distance between them travelling along a line through the centre of the nucleus is appreciably greater (5.5 μ). As metaphase proceeds the chromatin separates into discrete bivalents, each having a pair of kinetochores (Figures 42, 43 and 44).

viii) *Anaphase I*

Anaphase I kinetochores consist of at least 4 layers (Figure 45). The outermost layer (the most darkly staining region) is the one upon which the chromosomal microtubules converge (Figure 45). This darkly staining region overlays alternating electron transparent and scattering layers. As well as chromosomal microtubules, there exist polar microtubules which on occasion pass directly through a bivalent (Figure 44).

FIGURE 40. Higher magnification of Figure 39. Intranuclear microtubules (arrow heads) can be seen surrounded by chromatin (Ch) in the portion of the nucleus shown. Magnification 51,600. Bar = .5 μ

FIGURE 41. Model of an early metaphase I nucleus. This 3 dimensional reconstruction shows the shape of the nucleus at this time. The locations of polar fenestrae (Pf) and basal bodies (Bb) are indicated. An evagination (E) is also indicated. Magnification 21,000. Bar = 1 μ

Ch
40

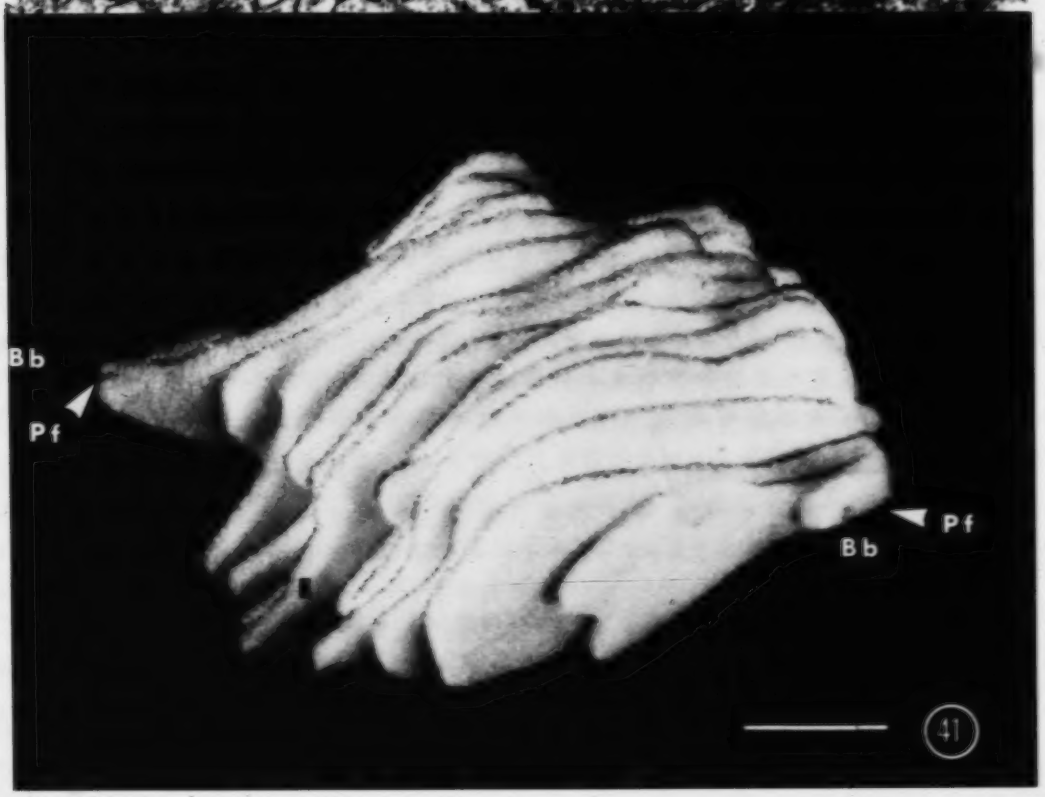

Bb
Pf
Bb
Pf
41

FIGURE 42. Late metaphase I to anaphase I cell fixed at 8½ hours. The nuclear envelope (Ne) lacks the evagination shown in Figure 41. Shown in the plane of this section is a polar fenestra (Pf). The cell wall (W) is considerably removed from the cell membrane (M). Magnification 12,200. Bar = 1 μ

FIGURE 43. Higher magnification of Figure 42. Microtubules (Mt) and kinetochores (K) are evident at this magnification. The ribosome free area (Rf) associated with the spindle pole is also indicated. Magnification 26,200. Bar = 1 μ

W
M
N
S
Pf
42
Pf

FIGURE 44. Anaphase I cell fixed at 8.75 hours. Shown in the plane of this section are 7 distinct kinetochores (arrows). Two bivalents (Bi) show both kinetochores. Also present are chromosomal microtubules (Mtc) and polar microtubules (Mtp). Magnification 33,800. Bar = .5 μ

FIGURE 45. Late metaphase I to anaphase I kinetochore. The kinetochore can be seen to consist of at least 4 layers. The microtubules' attachment to the outer layer (OL) is indicated by arrows. This layer is followed by an outer middle layer (OML), an inner middle layer (IML) and an inner layer (IL). Magnification 125,000. Bar = .2 μ

Bi
Bi
IML
Ch
OL
IL
45

At anaphase the nucleus becomes less hooked in shape than it was at metaphase. This is accomplished without a detectable increase in the pole-to-pole distance through the nucleus. As the cell proceeds into late anaphase/telophase the nucleus is now almost cylindrical (Figures 46 and 47), being approximately 4 μ long x 1.3 μ in diameter in this cell. The cylindrical shape is rounded at each pole, where a single spherical mass of condensed chromatin is found. The chromatin-free space between the poles of the nucleus is about 1 μ in length.

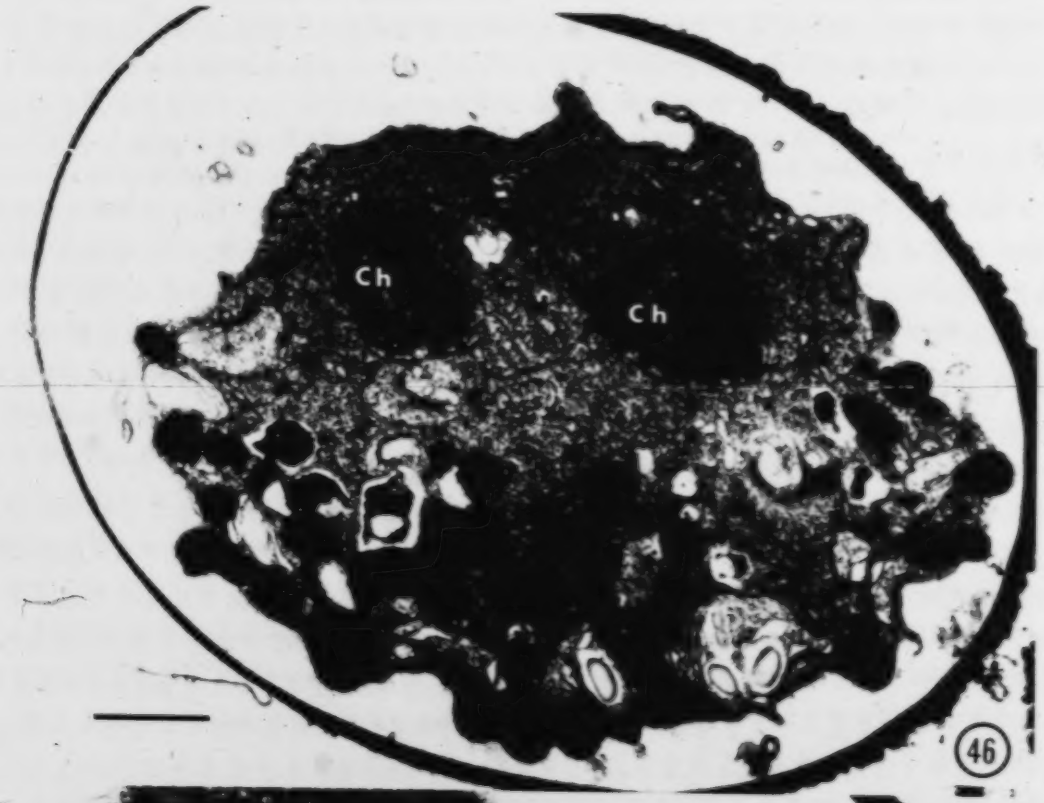
ix) *Cytokinesis*

Figure 48 shows a cell during cytokinesis with the two reconstituted nuclei considerably removed from one another. The chromatin is still condensed at this stage, with no nucleolus present. Remnants of the parent nucleus and interzonal spindle can be seen lying between the two nuclei (Figure 49). The two daughter nuclei are now completely bound by their own nuclear membranes, although the remnants of the parent nucleus can remain attached to the reformed nuclei (Figure 49).

As cytokinesis progresses the two daughter nuclei remain some distance apart. Between them lies the phycoplast system of microtubules and vesicles (Figure 49). It is along this line of microtubules and vesicles that the cleavage furrow is found. There are also microtubules concentrated at the apex of the cleavage furrow and along

FIGURE 46. Median section through a telophase cell fixed at 8.75 hours. The nucleus (N) is almost cylindrical in shape and is found to one side of the cell. Magnification 17,000. Bar = 1 μ

FIGURE 47. Higher magnification of Figure 46. This longitudinal section through a telophase nucleus shows chromatin (Ch) condensed at the ends of the nucleus. The parent nuclear envelope (Ne) still joins the two nuclei. Magnification 31,900. Bar = 1 μ


Ch
Ch
46

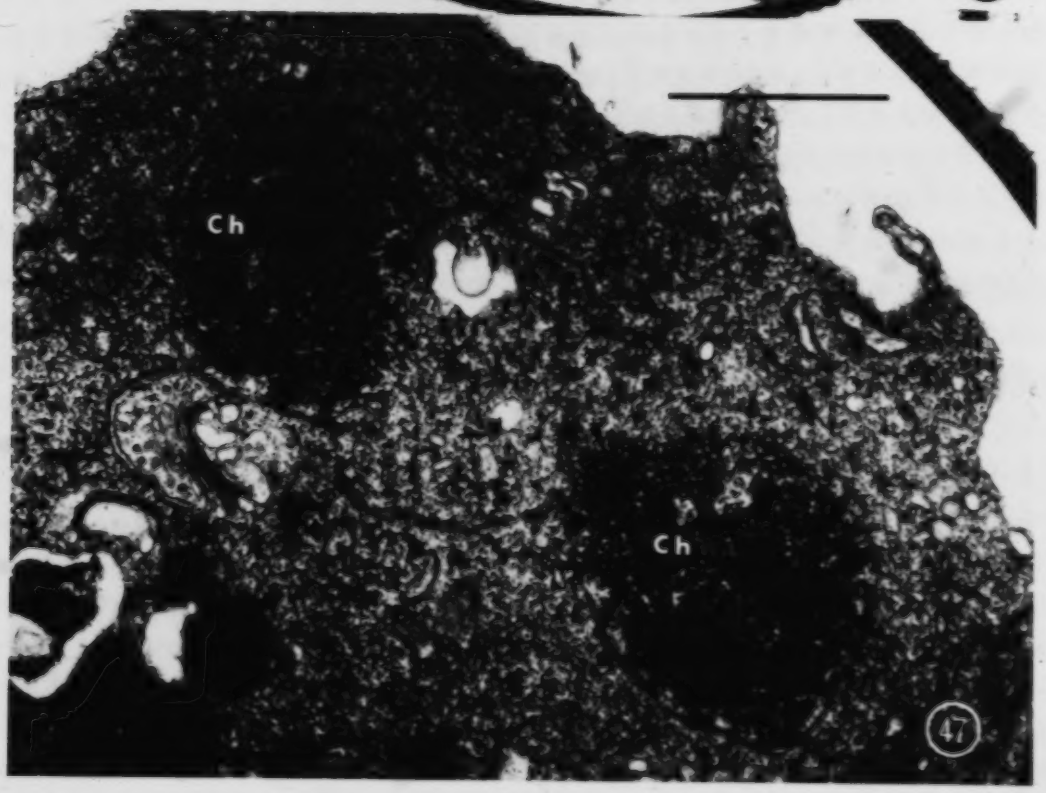
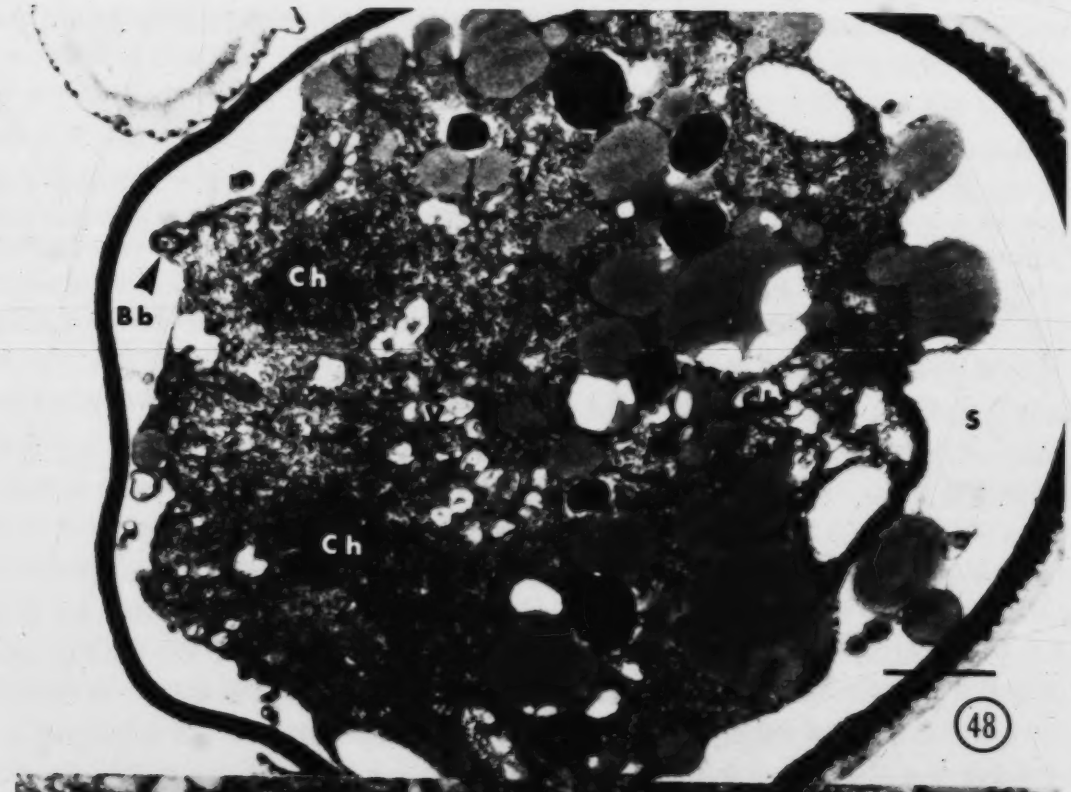
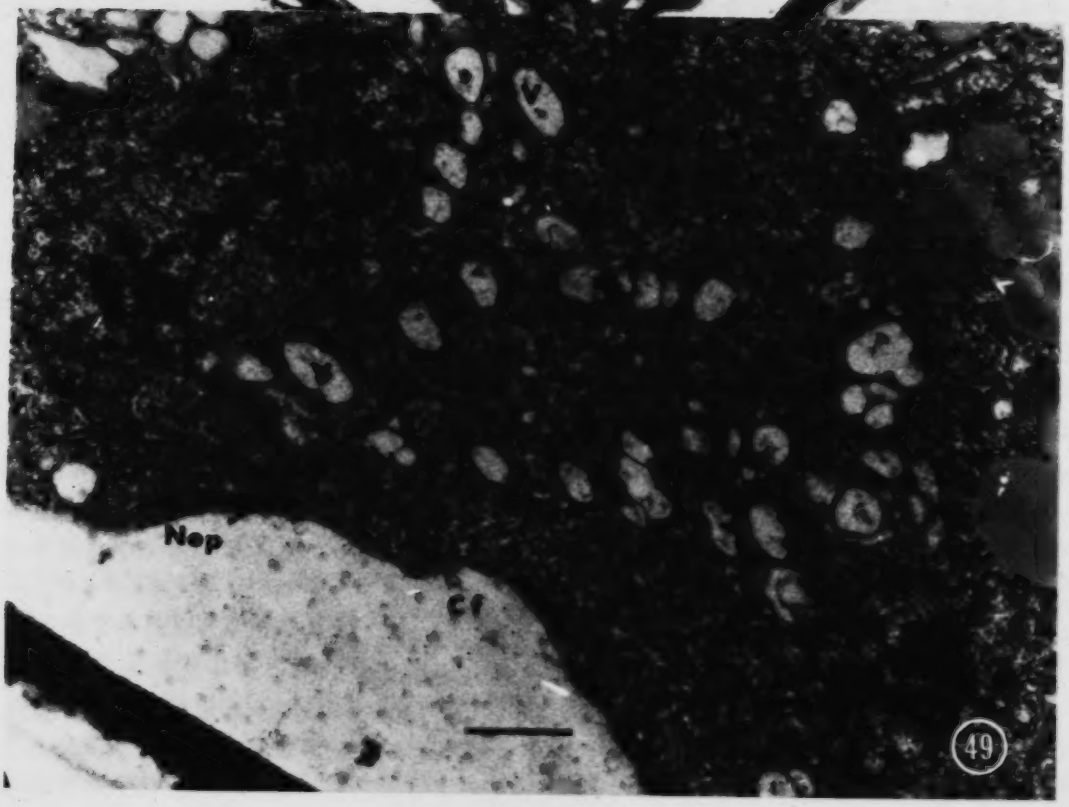

Ch
Ch
47

FIGURE 48. Median section of a cytokinesis cell fixed at 8.75 hours. The two nuclei have separated. The chromatin (Ch) remains condensed. The two nuclei are separated by some distance. A basal body (Bb) is present and a row of vesicles (V) is found along a plane which passes between the nuclei. Magnification 15,900. Bar = 1 μ

FIGURE 49. Higher magnification of a median section through a cell during cytokinesis. Vesicles (V) lie between the nuclei. The cleavage furrow (CF) has started to form. A portion of the parent nuclear envelope (Nep) can be seen attached to the upper nucleus. Magnification 62,300. Bar = .25 μ


Bb
Ch
Ch
S
48


Nop
Cf
49

its edge (Figure 50). Figure 51 also shows microtubules radiating out from the basal body pairs parallel to the cleavage furrow.

Figure 52 shows a zygospore with a nearly completed cleavage furrow. Lying in the path of the cleavage furrow is a group of 4 basal bodies. It is not known whether these represent a basal body pair recently duplicated in preparation for the 2nd meiotic division, or the original two basal bodies of the first division zygospore.

x) *Interkinesis*

Figure 53 shows a diad with a nearly completed cleavage furrow. As can be seen in this figure the nucleus of the upper product of meiosis I lies toward the side of the daughter cell opposite the chloroplast. Examination of Figures 53 and 56 and of sections through many other diads at interphase and metaphase II indicates that the meiosis I products are at right angles to one another. Thus, for example, the plane of the section in Figure 53 through the lower product is believed to be that expected if reference planes (*i.e.* planes representing a constant morphological aspect) for the two daughter cells are at right angles to each other. Each nucleus contains a single nucleolus together with small amounts of condensed chromatin scattered throughout.

FIGURE 50. The edge of an interphase II diad cleavage furrow. The cleavage furrow (CF) has microtubules (arrows) associated with it. Magnification 118,000. Bar = .2 μ

FIGURE 51. Interphase II diad fixed 8.75 hours after the onset of germination. The cleavage furrow (CF) has almost separated the two cells. The chromatin has decondensed and the nucleolus (Nu) has reformed. The basal bodies (Bb) of both cells are found adjacent to each other next to their respective nuclei. Microtubules (arrows) can be seen radiating out from the basal body pairs parallel to the cleavage furrow. Magnification 41,700. Bar = .5 μ

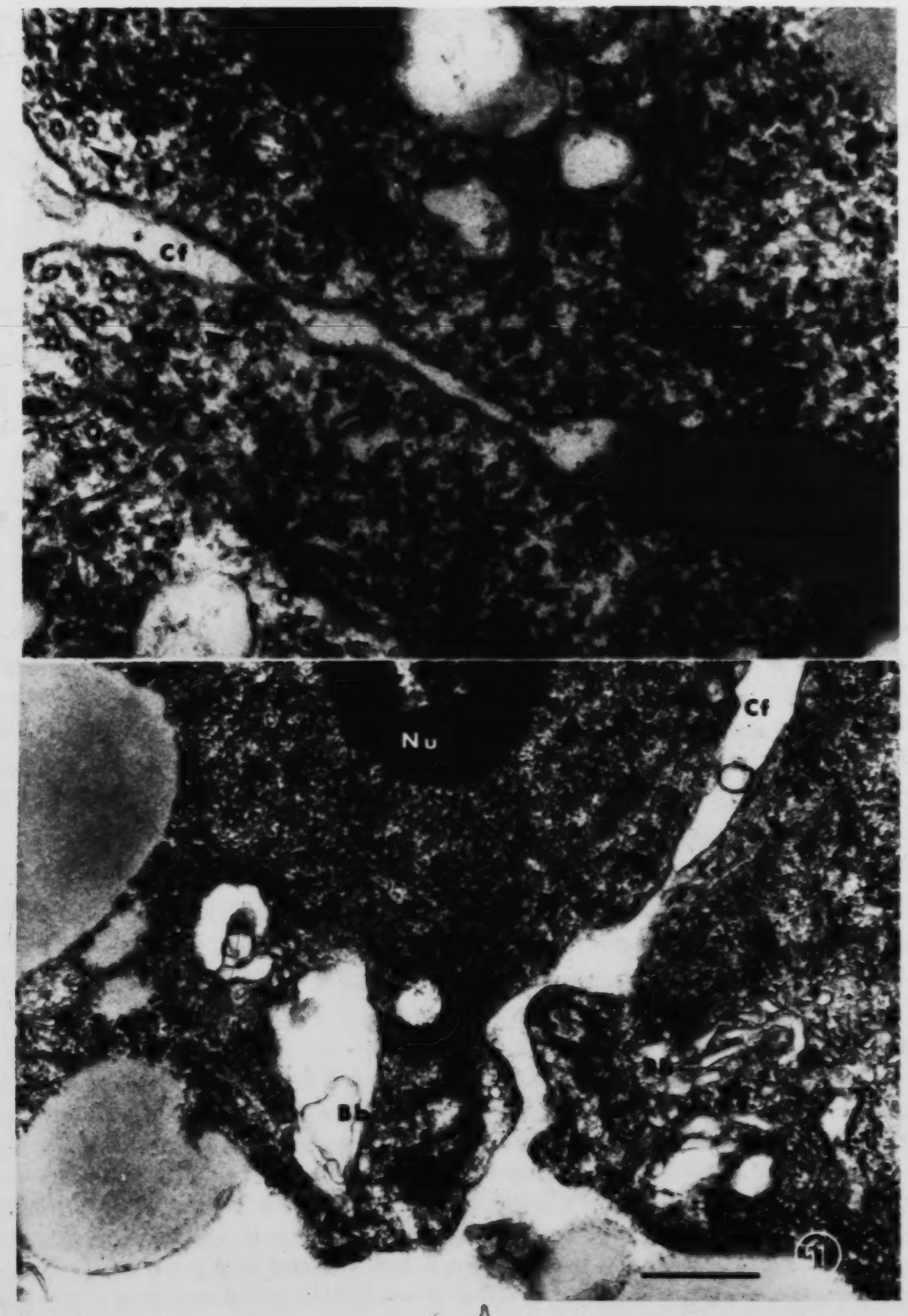
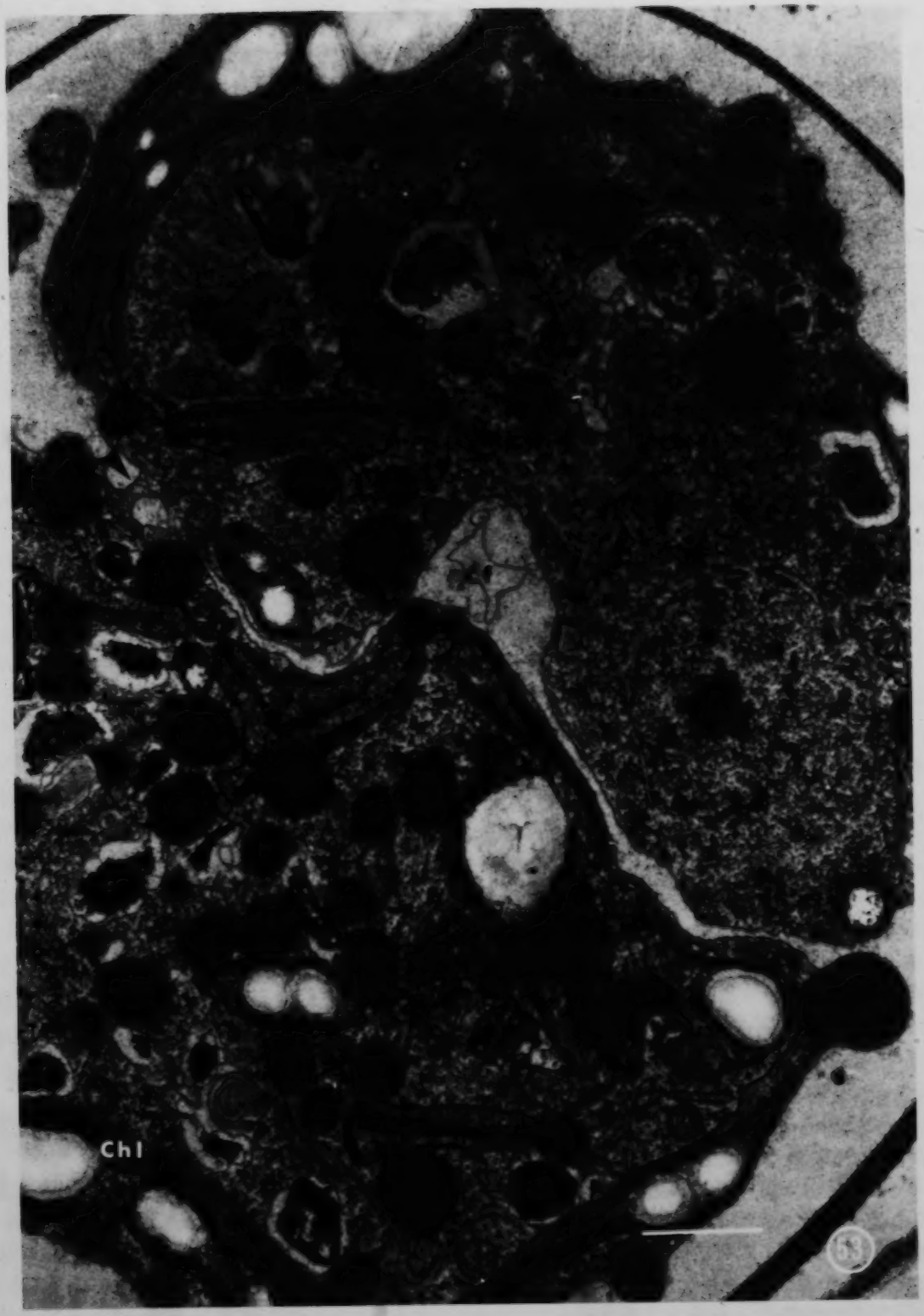

Cf
Nu
Cf
Bb
Bf

FIGURE 52. A cell fixed at 9 hours. A group of 4 basal bodies (Bb) lies in the path of the cleavage furrow (CF). Magnification 14,100. Bar = 1 μ

Cf
Cf
52

FIGURE 53. Diad fixed at 8.75 hours. Included in the plane of the section is an interphase nucleus (N). The cleavage furrow (CF) almost separates the two products with only a narrow neck (arrow) joining them. Magnification 21,000. Bar = 1 μ


Chl

xi) *The 2nd meiotic division*

The second division is very similar to the first, except that the nuclei and kinetochores are smaller and less striking. Figure 45 shows a first division kinetochore and Figure 55 shows a second division kinetochore at the same magnification. Both kinetochores can be seen to consist of at least 4 layers, although the maximum size observed along the major axis in 2nd division kinetochores is considerably reduced. Figure 57 shows a 2nd division cell undergoing cytokinesis. All three nuclei within the plane of the section are in interphase and each contains a single nucleolus. Figure 58 shows the 4 meiotic products contained within the parent cell wall; all the products contain extensive mature chloroplasts and interphase nuclei contain a single nucleolus.

B. Timing of Meiotic Events

In *Chlamydomonas reinhardi* synchronous meiosis may be obtained by transferring zygotes, stored in the dark, to light for a one hour light pulse. The zygospores then undergo the two meiotic divisions. The two meiotic divisions are sometimes immediately followed by a mitotic division, depending on the strain used (Tan and Hastings, 1977).

For the construction of stage distribution curves, samples are removed from the germinating culture at half hour or three-quarter hour intervals and prepared for

FIGURE 54. Longitudinal section through a late metaphase II anaphase II nucleus. The chromatin (Ch) has condensed into individual chromosomes with their associated kinetochores (arrows). Both polar fenestrae (Pf) are in the plane of this section. The ribosome free area (Rf) near the lower pole is probably associated with the basal bodies at that pole. Magnification 27,000. Bar = 1μ

FIGURE 55. Anaphase II kinetochores (K) with associated microtubules (Mt). The kinetochores are made up of 4 layers as were the 1st division kinetochores. Magnification 125,000. Bar = .25 μ

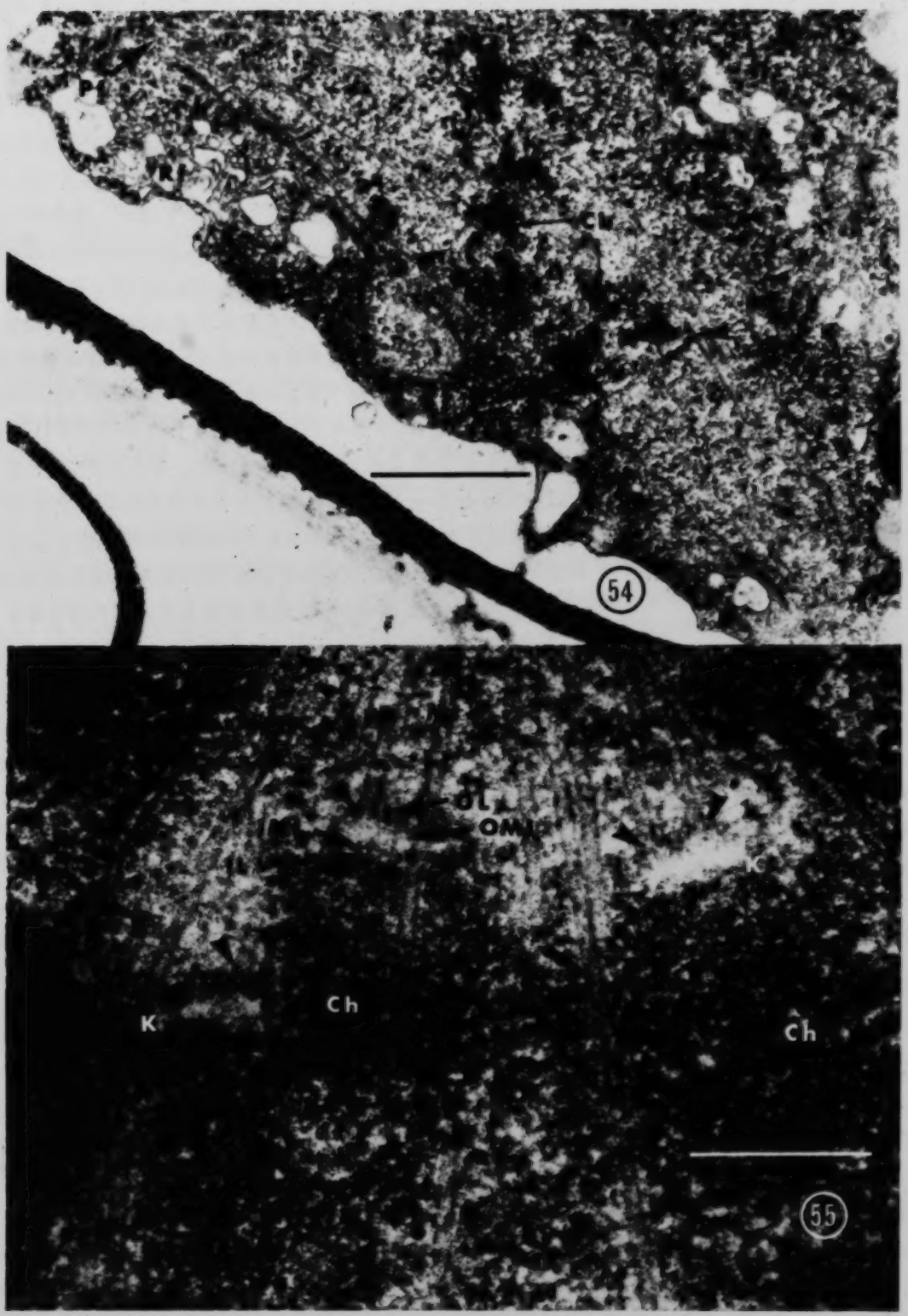

PI
PI
CM
54
OL
OM
IL
C
K
Ch
Ch
55

FIGURE 56. Diad fixed at 9 hours. The two first division products are separated. The plane of the section passes through the nuclei (N) of both products. The upper nucleus is in early anaphase II. Several chromosomes (Chr) are present in the upper nucleus. Some chromosomes show kinetochores (arrows). A basal body (Bb) is present adjacent to the polar fenestra (Pf) of the upper nucleus. The spindles of the two nuclei are at right angles to each other. Magnification 13,000. Bar indicates 1 μ

FIGURE 57. Section through a cell fixed at 9 hours. Three interphase nuclei (N) are present in the plane of the section. The two products on the right of the micrograph have not yet been separated by the cleavage furrow (CF). Magnification 13,000. The bar indicates 1 μ

Bb
Ch
D
Sp
56
CF
57

FIGURE 58. Tetrad from a sample fixed at 10 hours. Shown in the plane of the section is a nucleus (N) at interphase. An extensive space (S) separates the meiotic products from the parent wall (W). A well defined chloroplast (Chl) is present in all four products. Magnification 18,000. Bar represents 2 μ

S
Gr
Chl
St
Chl
W
58

analysis by light and electron microscopy. Two such experiments were carried out, one using a zygote preparation which was heterozygous for two linked arginine requirements in the trans configuration, and one which was wild type.

The strain heterozygous for the two arginine requirements was used to determine the degree of synchrony and the duration and timing of the different stages by scoring with the electron microscope. Approximately 600 cells were scored for each time point, care being taken to ensure that each section was approximately the same thickness. Scoring of stages other than diads or tetrads required that the cells be sectioned through the nucleus. This occurred approximately 42% of the time. The total cell membrane-bound volume of the zygospores remained essentially constant from the beginning of prophase to the formation of diads. The results obtained by scoring zygospores heterozygous for the two arginine requirements are given in Table II.

The duration of stages was estimated by using two methods. The first was carried out directly from Figure 59 a-g by calculating the total area represented by each stage in the histograms. The second method requires the assumption that the curve representing the probability of finding a cell in a given stage with time is a normal distribution curve. With this assumption, one may plot the percentage of cells that were at or beyond a given

TABLE II. Cytological stage data obtained by scoring samples of the strain heterozygous for the two arginine requirements. Samples, taken at half hour intervals starting at 5.75 hours and finishing at 9.75 hours after the onset of germination, were sectioned and scored with the electron microscope. The number scored column refers to the total number of cells scored. The columns headed unexpanded zygospores, expanded zygospores, diads and tetrads indicate the number of zygospores which fell into each of these categories. The columns headed leptotene through anaphase/telophase give the number of cells which fell into each of these cytological stages at each of the sampling times. These cytological stages are the subdivisions of the expanded zygospore class. 42% of the expanded zygospores were sectioned through the nucleus enabling this further classification.

Time	Total Number of Zygospores Scored	Unexpanded Zygospores	Expanded Zygospores	Diads	Tetrads
5.75	456	168	288	-	-
6.25	436	123	313	-	-
6.75	591	91	499	-	1
7.25	748	41	707	-	-
7.75	724	-	700	20	4
8.25	649	-	602	39	8
8.75	593	-	440	106	47
9.25	541	-	313	137	91
9.75	284	-	96	83	105

TABLE II

Time	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Metaphase	Anaphase
5.75	16	21	3	-	-	-	-
6.25	19	35	4	-	-	-	-
6.75	35	85	13	-	-	-	-
7.25	49	111	76	7	2	2	1
7.75	10	36	163	29	6	1	1
8.25	4	13	88	62	11	9	2
8.75	2	5	42	40	10	8	1
9.25	2	-	13	44	40	20	5
9.75	2	-	11	17	24	1	-

TABLE II continued

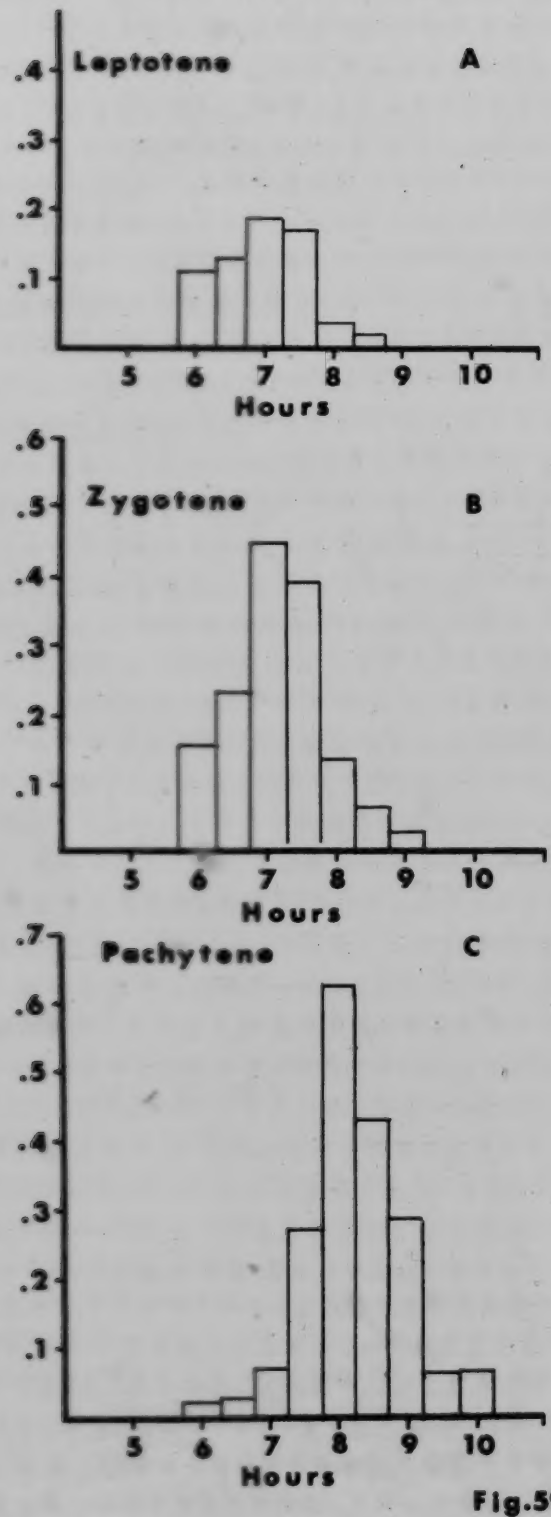
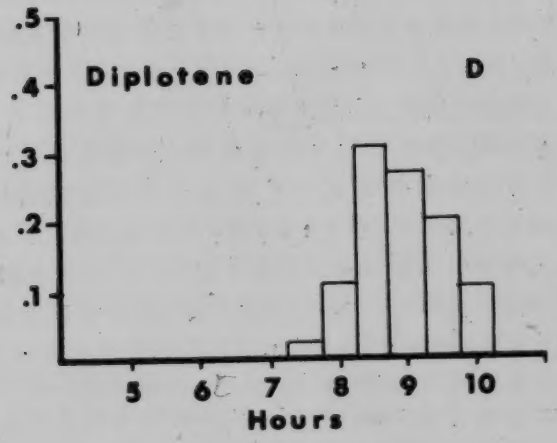

Leptotene
A
.4
.3
.2
.1
5
6
7
8
9
10
Hours
Zygotene
B
.6
.5
.4
.3
.2
.1
5
6
7
8
9
10
Hours
Pachytene
C
.7
.6
.5
.4
.3
.2
.1
5
6
7
8
9
10
Hours

Fig.59


.5
.4
.3
.2
.1
Diplotene
D
5
6
7
8
9
10
Hours

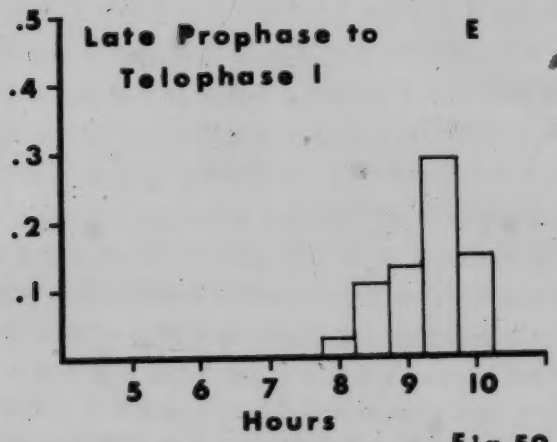

.5
.4
.3
.2
.1
Late Prophase to
Telophase I
E
5
6
7
8
9
10
Hours

Fig.59

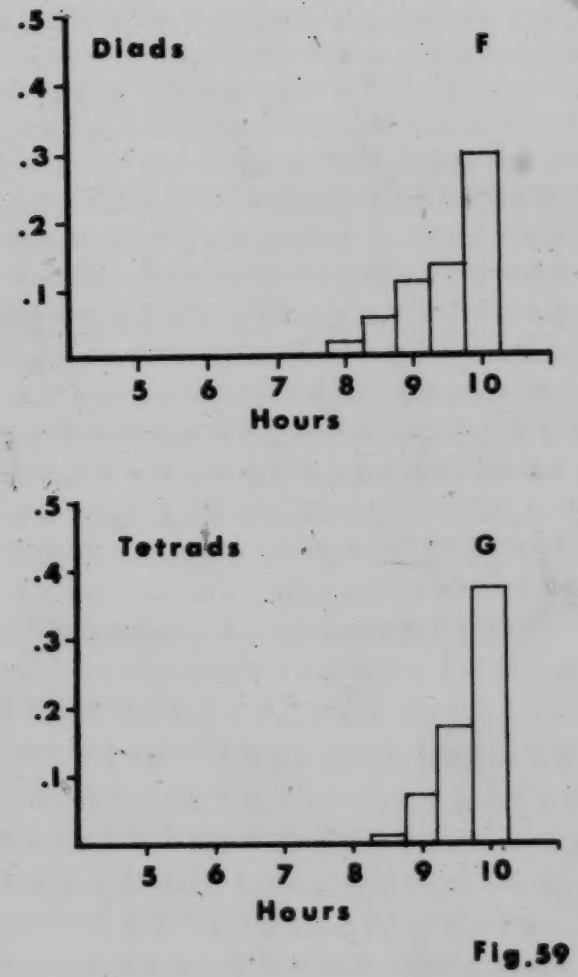


FIGURE 59 A-G

Histogram stage distribution curves for the strain heterozygous for the two arginine requirements. Abscissa: time after the onset of germination (hours); ordinate: proportion of cells. Stage distribution: *i.e.* proportion of cytological stages found at different times during germination. A - Leptotene; B - Zygotene; C - Pachytene; D - Diplotene; E - Late Prophase-Telophase I; F - Diads; G - Tetrads.

stage against time on probability paper. If the data fits a normal distribution, the data curve will be a straight line on probability paper. Although the data points scatter about a straight line, a line representing the best fit to the data can be obtained by the method of Finney (1971). Once the best line has been obtained for each stage of interest it is possible to obtain an estimate of the durations of the different stages by measuring how long it takes for 50% of the cells to pass from the stage of interest into the next stage. The graphs produced using this analysis of the arginine heterozygotes are presented in Figure 60.

The durations of the different stages, as calculated by the two methods, are given in Table III. Also obtainable from the two methods are estimates of the times at which the maximum proportion of cells are in given stages, also given in Table III.

The plots on probability paper have the added advantage that an arbitrary estimate of the degree of synchrony can be obtained from them. One method, the "probit analysis" method, is to estimate the time necessary for the population to go from 2.5% to 97.5% in the same stage or later. The estimates of the degree of synchrony are also given in Table III, and as shown, they vary from 3.6 hours for zygotene to 2.6 hours for pachytene. A final advantage of this method is that it enables one to

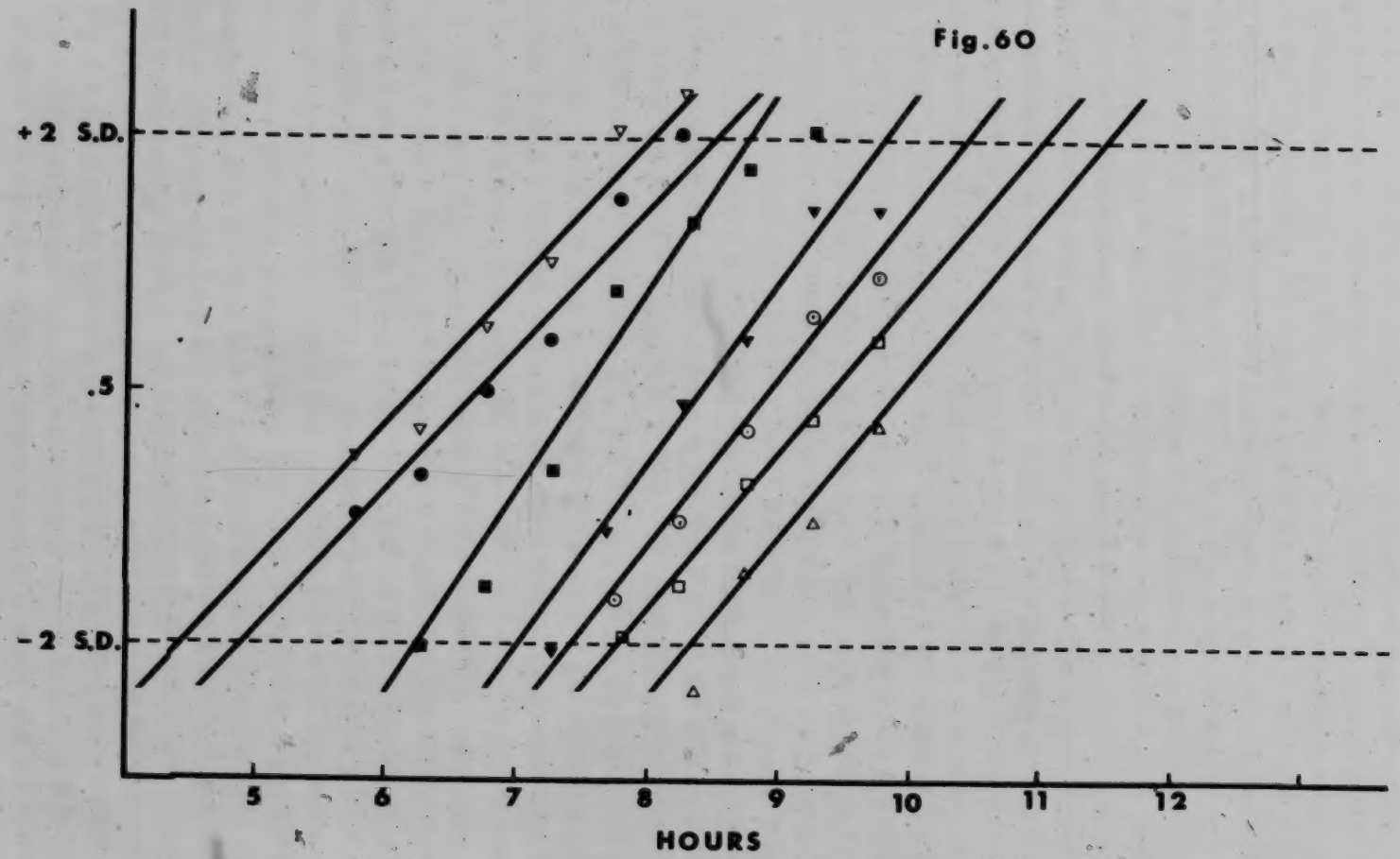


FIGURE 60. Entrance function curves for the strain heterozygous for the two arginine requirements fitted to the data in Table II by the method of Finney (1971). Abscissa: time after the onset of germination (hours); ordinate: standard deviations (S.D.). Leptotene ▽ ; Zygotene ● ; Pachytene ■ ; Diplotene ▼ ; Late Prophase-Telophase I ○ ; Diads □ ; Tetrads △ .

Cytological Stage	Stage Duration Fig. 59	Stage Duration Fig. 60	50% Entrance Function Fig. 60	Stage Maximum Fig. 59	Stage Maximum Fig. 60	Synchrony ± 2 σ Fig. 60	Chi^2 and Degrees of Freedom Fig. 60
Leptotene	.41 hr.	.39 hr.	6.3 hr.	6.75 hr.	6.5 hr.	3.5 hr.	35*** 6
Zygotene	.73 "	.78 "	6.7 "	6.75 "	7.1 "	3.6 "	33*** 6
Pachytene	.92 "	.92 "	7.5 "	7.75 "	7.9 "	2.6 "	59*** 6
Diplotene	.51 "	.52 "	8.4 "	8.25 "	8.7 "	2.8 "	21*** 4
Late Prophase-Telophase I	.36 "	.46 "	8.9 "	9.25 "	9.1 "	3.0 "	5 3
Diads	- "	.52 "	9.4 "	- "	9.6 "	3.3 "	1.9 3
Tetrads	- "	- "	9.9 "	- "	- "	3.2 "	1.3 3

TABLE III. Results obtained from the histogram (Figure 59) and probability paper (Figure 60) plots for the arg 1 and arg 2 heterozygous strain.
$p < .1$ *; $p < .01$ **; $p < .001$ ***

estimate the duration of stages at the end of meiosis for which incomplete data is available. By the extrapolation of diad and tetrad frequency data, it was possible to estimate the duration of the diad stage to be .52 hours using this method.

The duration of meiosis in *Chlamydomonas* from leptotene to the end of telophase I was estimated to be 2.9 hours by the histogram area analysis and 3.1 hours by the probit analysis. Only the probit analysis enables one to estimate when meiosis begins and ends. Using the time when 50% of the cells have entered prophase as the beginning, and that when 50% of the cells are tetrads as the end, one can estimate the total duration of the meiotic divisions. By 6.3 hours after transfer to the light, 50% of the cells have entered prophase, and by 9.9 hours 50% of the zygospores are tetrads. This yields a total duration of 3.6 hours for the meiotic process in *Chlamydomonas*. Thus first prophase accounts for about 75% of the total meiotic division time. Pachytene was found to be the longest single stage by both methods of estimation: 0.92 hours by the histogram and 0.92 hours by the probit analysis. Diakinesis, metaphase, anaphase and telophase I were the shortest stages. Due to their short duration, very few observations fell into any of these classes. This made it advisable to lump these stages together and treat them as a single stage for purposes of time estimation.

These four stages had a total duration of .46 hours as estimated by the probit analysis and .36 hours as estimated by the histogram method.

After defining the meiotic stages of the arginine heterozygotes by electron microscopy, a second estimation of the duration and synchrony of stages was carried out on a wild type strain. This time, 1 μ sections of material prepared for electron microscopy were stained with toluidine blue and scored using a light microscope. Between 1,000 and 4,000 cells were scored for each time point, and analysis of the data was carried out as for the arginine strains. Scoring of stages other than diad or tetrad required that cells be sectioned through the nucleus. Cells in the first division of meiosis were sectioned through the nucleus 20% of the time. As was the case for the arginine zygospores, the total volume remained constant from the beginning of prophase until the formation of diads. The results obtained upon scoring wild type zygospores are presented in Table IV. The histograms obtained from these data are presented in Figure 61, and the plots on probability paper using the method of Finney (1971) are presented in Figure 62.

The lengths of the different stages for wild type zygospores, as calculated by the histogram and probit analysis methods, are given in Table V. Also present in Table V are the estimated times when each stage is at its maximum as estimated by the two methods.

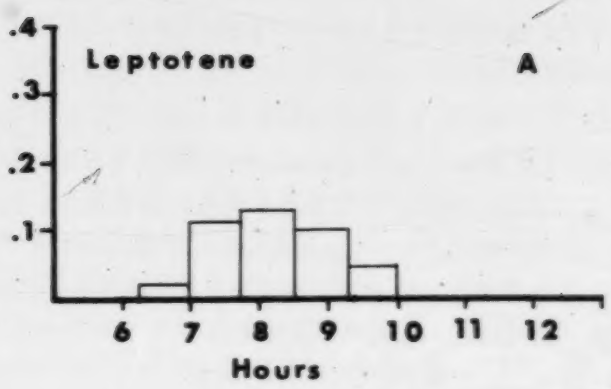
TABLE IV. Cytological stage data obtained by scoring samples of the 137C strain. Samples, taken at three-quarter hour intervals starting at 5½ hours and finishing at 11½ hours after the onset of germination, were sectioned and scored with the light microscope. The number scored column refers to the total number of cells scored. The columns headed unexpanded zygospores, expanded zygospores, diads and tetrads indicate the number of zygospores which fell into each of these categories. The columns headed leptotene through anaphase/telophase give the number of cells which fell into each of these cytological stages at each of the sampling times. These cytological stages are the subdivisions of the expanded zygospore class. 20% of the expanded zygospores were sectioned through the nucleus enabling this further classification.

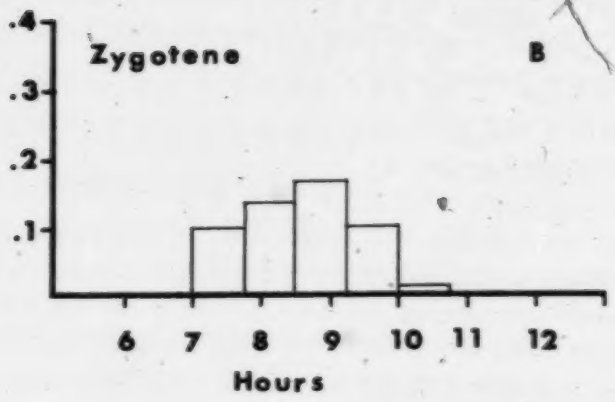
Time	Total Number of Zygospores Scored	Unexpanded Zygospores	Expanded Zygospores	Diads	Tetrads
5.5	914	895	19	-	-
6.25	1047	968	79	-	-
7.0	1888	1207	681	-	-
7.75	1736	782	952	1	1
8.5	3446	618	2808	12	8
9.25	1703	133	1538	21	11
10.0	1783	41	1488	164	90
10.75	1025	-	538	140	347
11.5	1337	-	396	199	742

TABLE IV

Time	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Metaphase	Anaphase
5.5	2	-	-	-	-	-	-
6.25	16	4	1	-	-	-	-
7.0	76	71	16	-	-	-	-
7.75	66	67	45	5	2	1	-
8.5	54	88	152	31	21	3	1
9.25	14	27	121	29	13	9	3
10.0	2	5	127	52	41	25	1
10.75	3	2	60	41	25	15	4
11.5	2	1	35	48	26	11	5

TABLE IV continued


.4
.3
.2
.1
Leptotene
A
6 7 8 9 10 11 12
Hours


.4
.3
.2
.1
Zygotene
B
6 7 8 9 10 11 12
Hours

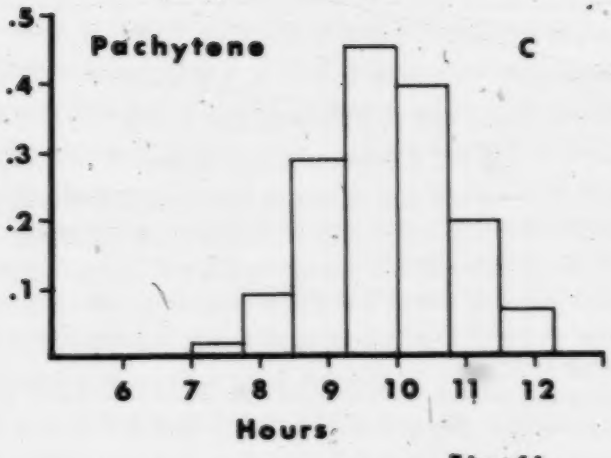
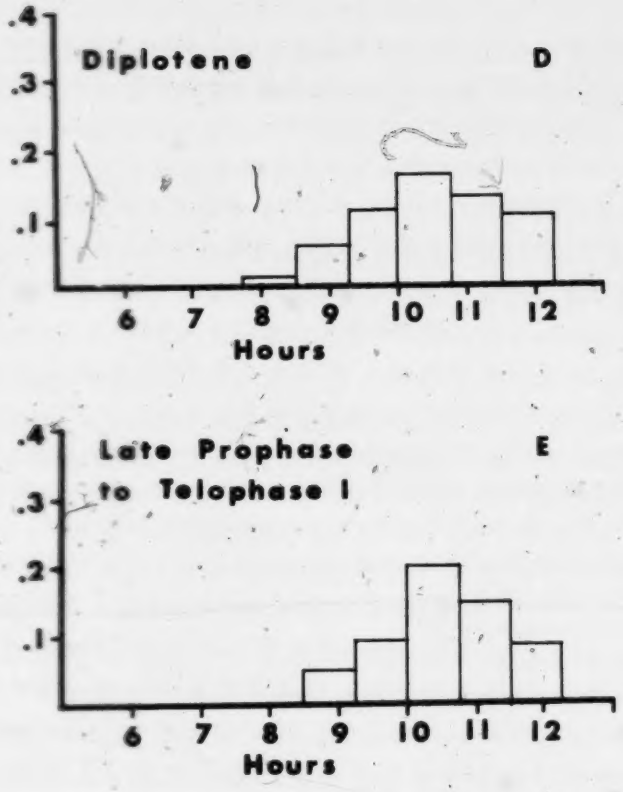

.5
.4
.3
.2
.1
Pachytene
C
6 7 8 9 10 11 12
Hours

Fig. 61


.4
.3
.2
.1
Diplotene
D
6
7
8
9
10
11
12
Hours
.4
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.1
Late Prophase
to Telophase I
E
6
7
8
9
10
11
12
Hours

Fig.61

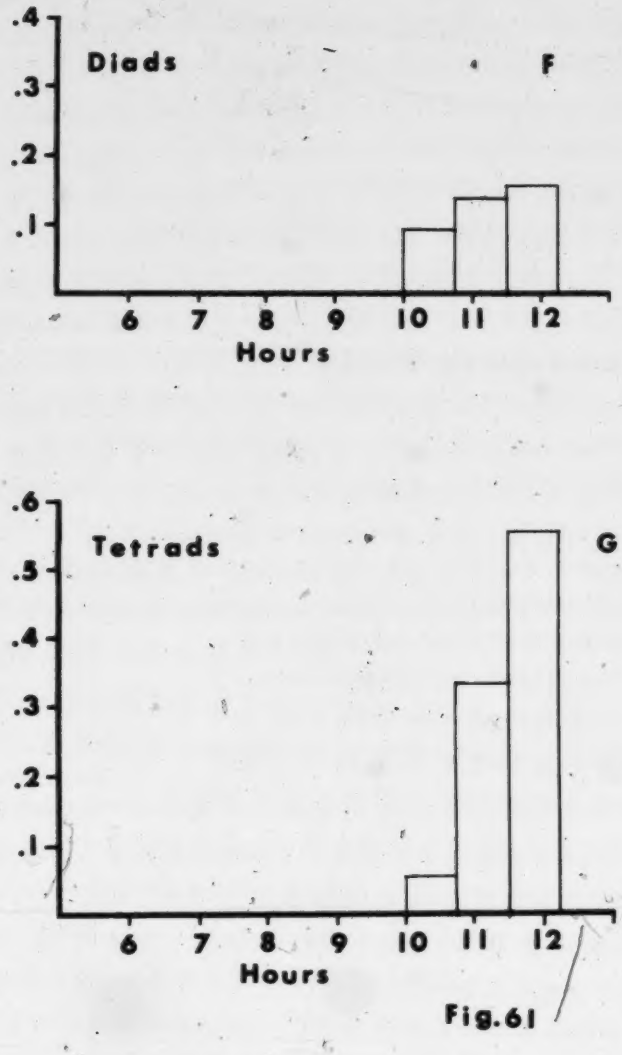

FIGURE 61 A-G

Histogram stage distribution curves for the wild type strain. Abscissa: time after the onset of germination (hours); ordinate: proportion of cells. Stage distribution: *i.e.* proportion of cytological stages found at different times during germination. A - Leptotene; B - Zygotene; C - Pachytene; D - Diplotene; E - Late Prophase-Telophase I; F - Diads; G - Tetrads.

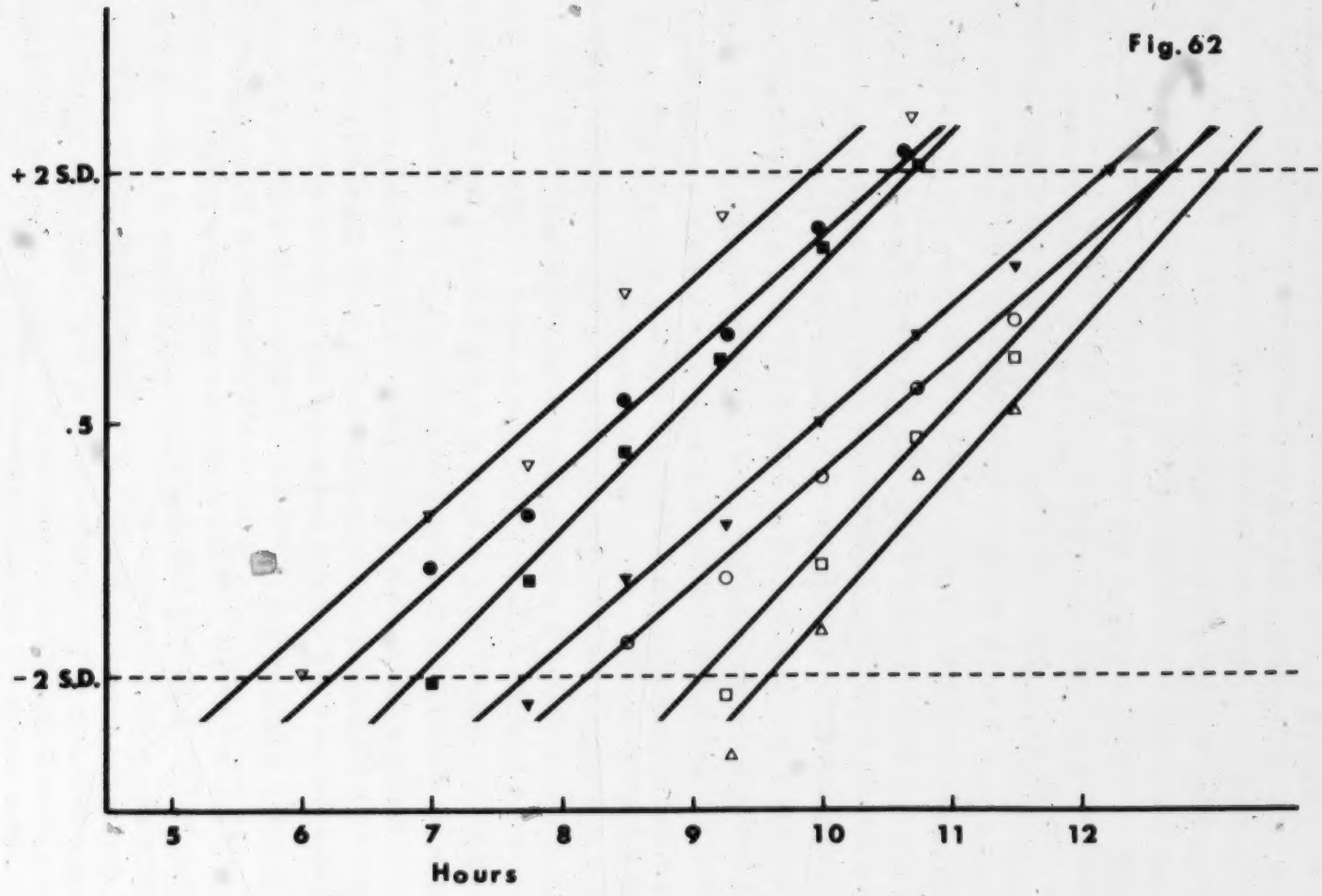

FIGURE 62. Entrance function curves for the wild type strain fitted to the data in Table IV by the method of Finney (1971). Abscissa: time after the onset of germination (hours); ordinate: standard deviations (S.D.). Leptotene ▽ ; Zygotene ● : Pachytene ■ ; Diplotene ▼ ; Late Prophase-Telophase I ○ ; Diads □ ; Tetrads △ .

Cytological Stage	Stage Duration Fig. 61	Stage Duration Fig. 62	50% Entrance Function Fig. 62	Stage Maximum Fig. 61	Stage Maximum Fig. 62	Synchrony ± 2 σ Fig. 62	Chi^2 and Degrees of Freedom Fig. 62
Leptotene	.34 hr.	.40 hr.	7.8 hr.	7.75 hr.	8.0 hr.	4.3 hr.	114*** 7
Zygotene	.40 "	.42 "	8.4 "	8.5 "	8.6 "	4.3 "	56*** 6
Pachytene	1.14 "	1.17 "	8.8 "	9.25 "	9.4 "	3.7 "	49*** 6
Diplotene	.43 "	.49 "	10.0 "	10.0 "	10.2 "	4.5 "	5.9 4
Late Prophase-Telophase I	.44 "	.43 "	10.5 "	10.0 "	10.7 "	4.5 "	5.8 4
Diads	- "	.45 "	10.9 "	- "	11.1 "	3.5 "	8.7 4
Tetrads	- "	- "	11.3 "	- "	- "	3.6 "	15** 4

TABLE V. Results obtained from the histogram (Figure 61) and probability paper (Figure 62) plots for the germination of the 137C strain.
$p < .1$ *; $p < .01$ **; $p < .001$***.

The synchrony estimates obtainable from the probit analysis indicate that the wild type zygospores proceeded through meiosis less synchronously than the arginine zygospores. The degree of synchrony, *i.e.* the time required for 50% ± 2σ to pass through a given stage, varied from a high of 4.5 hours for diplotene to a low of 3.5 hours for diads.

Using the same criteria for time of entrance into meiosis and time of completion that was used before, it is found that meiotic prophase begins at 7.8 hours and meiosis is completed at about 11.3 hours. This yields an estimate of 3.5 hours as the total duration of meiosis. The longest meiotic stage was again found to be pachytene; its length was estimated to be 1.2 hours by probit analysis and 1.1 hours by the histogram method. Pertinent data on stage duration, degree of synchrony and time of stage maximum are given in Table V.

Chiu (1973) showed that there is a peak of ^{32}P incorporation into total DNA at an early stage in the germination of *Chlamydomonas*. He was also able to show that FUdR, when pulsed at this time, caused a decrease in recombination. Since then, Tan and Hastings (1977) have shown that the ^{32}P incorporation peak coincides with the time of the main pre-meiotic DNA synthetic period, as determined by density shift experiments. It is therefore possible to estimate indirectly the time of the pre-meiotic S period in the germination of the arginine zygospores.

This is done by determining when FUdR-induced depression in the level of recombination occurs relative to the times of stage maximum and duration of the different meiotic stages as estimated by the histogram and probit analysis methods. Samples were taken from the same population of cells as were those fixed for analysis by electron microscopy, and given half hour pulses of .2 mM FUdR. Examination of Figure 63, which shows levels of recombination plotted against time, shows that the early FUdR effect was greatest at 5½ hours. This means that the peak of DNA synthesis occurs approximately 1 hour before the stage maximum for leptotene.

The time of the main pre-meiotic S period was also estimated directly using the wild type strain. Figure 64, reproduced with the permission of Dr. C. K. Tan and Dr. P. J. Hastings, shows a graph of $^{32}PO_4$ pulse labelling into the total DNA of *Chlamydomonas* zygospores. The samples for this pulse labelling experiment were taken from the same population of cells as were the samples for Figures 61 and 62. The results of this experiment show that the pre-meiotic DNA synthetic period peaks at between 5 and 6 hours. The peak of the pre-meiotic DNA S period in this strain precedes the point where 50% of the cells have entered prophase by 1.5 hours.

FUdR also causes an increase in recombination when pulsed at a time separate from the effect mentioned earlier. This effect occurs during prophase (Figure 63).

Fig. 63

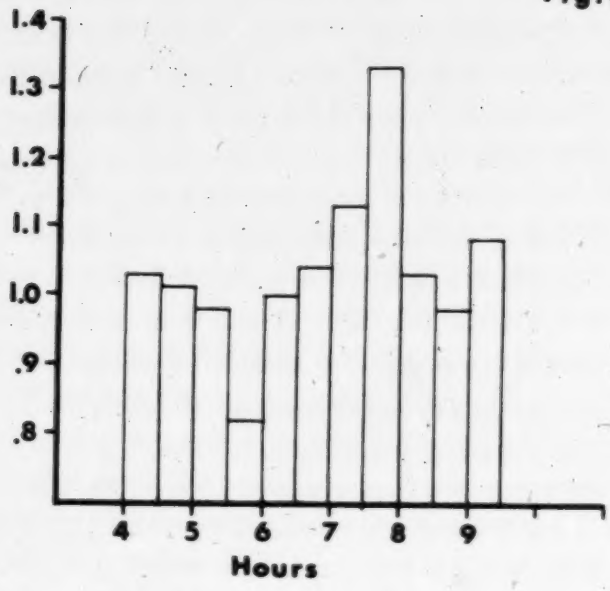

FIGURE 63. Recombination between arg-1 and arg-2 as a proportion of the control value following one-half hour treatments with 2 mM FUdR. Samples treated were from the same culture as those used to obtain the data in Table II.

Hour treatment	Total colonies	% recombinant tetrads
4 - 4.5	1960	24.8
4.5 - 5	1376	24.8
5 - 5.5	1303	23.8
5.5 - 6	1968	19.9
		$\chi^2 = 14.8$***
6 - 6.5	1705	24.3
6.5 - 7	1747	25.3
7 - 7.5	1927	27.5
		$\chi^2 = 7.46$**
7.5 - 8	1819	32.5
		$\chi^2 = 41.0$***
8 - 8.5	2142	23.8
8.5 - 9	1528	25.1
9 - 9.5	1934	26.3
Control	4397	24.3

* $p < .05$

** $p < .01$

*** $p < .001$

TABLE VI. Recombination between *arg-1* and *arg-2* after treatment with 2mM FUdR at different times during zygospore germination. Contingency χ^2 values have 1 degree of freedom.

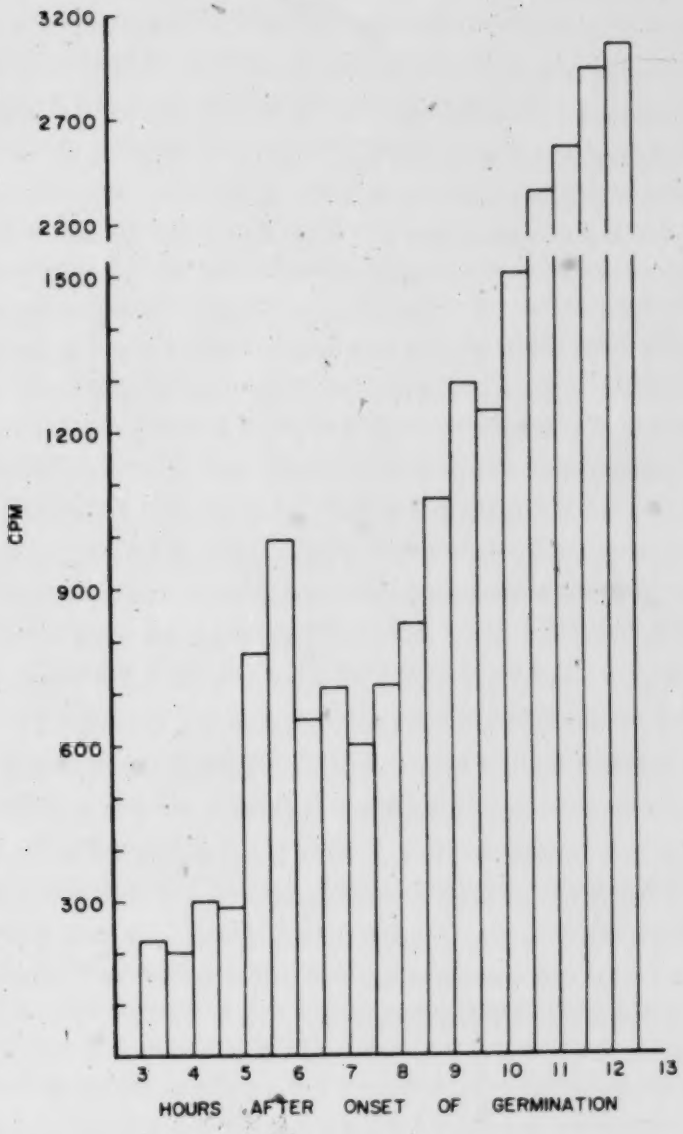

FIGURE 64. $^{32}PO_4$ incorporation into DNA during one-half hour pulses during meiosis of the 8-spored variant of strain 137C. Reproduced with the permission of Dr. C. K. Tan and Dr. P. J. Hastings. Abscissa: hours after the onset of germination; ordinate: counts per minute.

The maximum increase in recombination is produced when FUdR is pulsed for one half hour, beginning at 7½ hours. This increase corresponds with a time when 50% of the cells are in pachytene or later as estimated by the probit analysis (Figure 60) and better than 60% of the cells are in pachytene as estimated by the histogram method (Figure 59d). The stage maximum for pachytene is estimated to be 7.8 hours by the probit analysis method and 7.75 hours by the histogram method. Thus the stage maximum lies within the same half hour interval as does the largest enhancement of recombination levels by FUdR.

FUdR has been shown by Hastings (1964) and Chiu (1973) to be representative of a group of agents which alter levels of recombination at this time. This sensitive period is the latest period during meiosis at which recombination levels can be altered. Making the assumption that recombination events cannot take place, or at least have not been fixed, prior to the latest time when treatments can alter the levels of recombination, it is possible to point to pachytene as the time when recombination occurs, or at least when recombination events are fixed.

CHAPTER IV

DISCUSSION

The histogram method provides the best estimate of stage duration (Nordby and Nordby, 1976). Unfortunately, the mature meiotic products are released from within the parent cell wall making it impossible to collect a complete set of samples. Therefore for stages near the end of the meiotic process the histogram method cannot be used, and probit analysis, which only requires the production of a straight line, can provide stage duration estimates from an incomplete set of data.

The estimation of stage maxima by the histogram method cannot be more accurate than the interval between samples, while the probit analysis method yields an estimate which uses all the data points. The probit analysis method is the only one which enables an estimation of the degree of synchrony by other than an *ad hoc* method. Therefore the stage maxima and degree of synchrony measurements discussed will be those obtained using probit analysis.

Very little detail can be observed in the nuclei of zygospores in the early stages (Figure 1). The irregular outline of the nucleus and the presence of a single nucleolus are the only distinguishable features. Cells which have completed the initial phase of expansion still show very little nuclear detail. This lack of detail is probably

due to the high electron density of zygospores at this time. With further expansion leading to the formation of a space between the cell wall and cell membrane (Figure 3) basal bodies were observed. This expansion with its associated reduction in overall cytoplasmic density could improve the fixation and/or staining in a fashion which would enable their detection. Another possibility is that basal bodies are absent prior to expansion and are synthesized *de novo* at the time of this expansion.

The duration of the pre-meiotic stage was 7.8 hours in the culture using strain 137C (Table V) and 6.3 hours in the culture using the strain heterozygous for the two arginine requirements (TableIII). The ^{32}P incorporation experiment (Tan and Hastings, 1977) (Figure 64), using samples from the same culture used to produce the data in Table IV, indicates that the pre-meiotic S period occurs between 5 and 6 hours. Chiu (1973) has demonstrated that a pulse of FUdR causes a depression in the levels of recombination when administered at the pre-meiotic S period. Thus the time of the pre-meiotic S period can be determined indirectly by following the effects of FUdR pulses on recombination. The FUdR experiment (Figure 63) shows that recombination levels are depressed by a 5½-6 hour pulse. Figure 60, the entrance function plots of the same culture, show that 50% of the zygospores have not entered meiosis until 6.3 hours. Therefore in both cultures, the pre-meiotic S period precedes prophase I of meiosis. This observation

is consistent with that observed during meiosis in other organisms (see Introduction).

If one accepts the overwhelming evidence that pre-meiotic associations are not directly involved in recombination (see Introduction), it can be concluded that the alteration of recombination levels at S period in *Chlamydomonas* is by an indirect mechanism. This is consistent with and lends support to the hypothesis of Chiu and Hastings (1973). The mechanism proposed for this indirect effect of FUdR on recombination levels is that levels of recombination are proportional to the amount of Z-DNA synthesis. Treatment of zygospores with FUdR at the pre-meiotic S period inhibited DNA synthesis causing the total S period to be extended and enabling more replicons to be initiated. This would mean that fewer replicons are left until prophase. Because the amount of prophase synthesis is proportional to the amount of recombination, treatment at S causes a reduction in recombination. This is consistent with the observation that the pre-meiotic S period of many organisms is longer than the mitotic S period (see Introduction) leading up to meiosis and this extended nature is due to to a decrease in the number of initiation sites (Callan, 1972).

Knowing the synchrony and the stage maxima of the different meiotic stages, it is possible to determine when to collect samples of a given stage and to estimate the purity of the samples. This would be of value if one wanted

to carry out biochemical measurements. It is possible to determine the feasibility of an experiment similar to that carried out by Hotta and Stern (1971a) demonstrating that approximately 0.3% of the genome of *Lilium* was not replicated until zygotene. Such a demonstration requires that synthesis at prophase be uncontaminated by synthesis taking place in cells which are late in proceeding through S period. This would mean that a very small proportion of the zygotene-like DNA could be replicated in S such that it overlapped with actual Z-DNA synthesis if Z-DNA synthesis were to be detected.

Using the peak of ^{32}P incorporation as the stage maximum for S period (Figure 64) and using the slope of the entrance into leptotene as a measurement of the synchrony of S period cells (Figure 62), one can obtain an estimate of the proportion of cells still in the S period at any given time. One can do the same thing with the first post-meiotic DNA synthetic period using the tetrad curve (Figure 62). Extrapolation from the probit analysis curves shows that there is a 1.5 hour period between the two synthetic peaks when less than 2.5% of the zygospores or their meiotic products are undergoing replication. This period is between 8 and 9½ hours and contains the stage maximum (8.6 hours) for zygotene. Unfortunately, one has to be able to determine the stage maximum for zygotene. This would require constant reproducibility of both synchrony and stage duration from one replicate experiment to another. A second problem is

that only 20% of the zygospores can be expected to be in zygotene when the stage maximum occurs (Figures 59 and 61).

The above considerations and the fact that labelling of Z-DNA synthesis would require a pulse of about ½ hour make the feasibility of an experiment to detect Z-DNA synthesis marginal. Two things could be done to improve the system. One would be to use an isolate of 137C which went only to 4's. This would eliminate the contamination from the second round of replication. The other would be to improve the synchrony of the system by trying different conditions and strains.

The leptotene nuclei shown in Figures 3, 5 and 6 show many discrete patches of chromatin; many of these patches contain lateral component within them. Such nuclei do not show regions of synaptonemal complex formation. These features are consistent with the definition of Moens (1973a) and indicate that *Chlamydomonas reinhardi* enters prophase I of meiosis in a typical fashion.

Using the strain heterozygous for the arg-1 and arg-2 loci, the duration of leptotene was .41 hours with a stage maximum at 6.5 hours. Using the wild type strain, the stage duration was found to be .34 hours and the stage maximum to be 8.0 hours. The duration of leptotene does not seem to vary significantly depending upon the type of analysis used. The time of the stage maximum and the 50% entrance function do vary, with 50% of the population having entered meiosis by about 6.3 hours when using the arginine-requiring strain

and 7.8 hours when using the wild type strain. Although the time of entrance into meiosis differs between the two cultures, the overall duration was the same (3.6 hours). This difference can, in part, be accounted for simply by the difference in degree of synchrony between the two cultures.

As the zygospore proceeds through leptotene, the cell wall expands dramatically. This expansion breaks the outer cell wall and the inner wall becomes much thinner, suggesting that it stretches in order to accomodate this expansion (Figure 7 *vs* Figures 1, 2 and 3). The increased volume of the zygospore is accounted for mainly by a large space which forms on the opposite side of the nucleus adjacent to the chloroplast. It may be that this expansion of the inner volume is a mechanism whereby the parent cell wall is broken down to release the meiotic products. This hypothesis is further supported by the behavior of some isolates which appear to proceed through germination to form tetrads without the accompanying expansion. The products of these zygospores are never released.

The general cytological characteristics of zygotene in *Chlamydomonas* appear to be similar to zygotene stages of most other organisms (see Introduction for a description of zygotene in other organisms). Lateral components or axial cores like those present in Figures 8-12 are continuous at this stage. They terminate on the nuclear envelope

opposite the basal body in a region adjacent to a group of granules (Figure 24). It is not known whether these granules are involved in membrane attachment and/or synthesis or whether their presence adjacent to this portion of the nuclear envelope is merely fortuitous.

The actual pairing process appears to be similar to that described by Lu and Raju (1970) for *Coprinus*. Figure 11 shows the two lateral components separated by .25 - .3 μ, each having loops of chromatin running out perpendicular from it in both directions, although most of the chromatin has moved to what will be the exterior of the complex. Closing of the distance between the lateral components leading to the synapsed state leaves the chromatin in a U shape around each lateral component with the side facing the other lateral component free of chromatin (Figure 16).

Figure 12, also of a zygotene nucleus, shows the interaction of the nucleolus and an unpaired portion of a chromosome at this time. This and other similar observations suggest that the nucleolus is involved in synapsis in *Chlamydomonas*. Involvement of the nucleolus in synapsis has been reported in other organisms (von Wettstein, 1977).

The stage maximum of zygotene in the heterozygous arg-1 arg-2 culture was 7.1 hours with a stage duration of .73 hours. For the other culture (137C), the stage maximum was estimated to be 8.6 hours with a stage duration of .40 hours. As for leptotene, the stage maximum is about 1.5 hours later for the wild type strain. The estimates of

stage duration for the two cultures are significantly different, the most probable explanation being that zygotene is very difficult to distinguish from pachytene using the light microscope. Because of this, one tends to score zygotene cells as pachytene, a fact which is reflected in the coincident increase in the length of pachytene when scoring is done using a light microscope. Because of this, the value of .73 hours, which is calculated from the electron microscope material, is more reliable.

Pachytene pairing in *Chlamydomonas* does not show the characteristic synaptonemal complexes seen in pairing during prophase of meiosis in most organisms when fixed and stained by standard procedures. Instead, bivalents are found paired at about .12 μ as in other organisms but lacking a central element. It is however possible to fix and stain *Chlamydomonas* in a fashion which does enable the detection of a central component (Figure 28). This method (method II of Materials and Methods), which removes the cell wall 10 minutes after fixation in glutaraldehyde has begun, results in the detection of a central element. In light of the detection of typical synaptonemal complexes in *Chlamydomonas*, it is suggested that the earlier observation of Bråten and Nordby (1973) that the green alga *Ulva mutabilis* lacked a central component may also be the result of the fixation technique used.

Figure 24, the reconstruction of nucleus I, shows

that *Chlamydomonas* has a bouquet arrangement of its bivalents at pachytene although the reconstructions of nucleus II (Figure 25) and nucleus III (Figure 26) do not reveal the bouquet arrangement as obviously. This could be due to the orientation of the nucleus relative to the plane of sectioning or the lack of a bouquet arrangement. Careful examination of nucleus III shows that the bouquet arrangement is not present. If bouquet formation and dispersal are standard processes in *Chlamydomonas*, breakdown precedes basal body duplication and is not basal body mediated as suggested by Moens(1973b) for locust. Rasmussen (1976) suggested that the formation of nuclear vacuoles causes the dispersal. Although no vacuoles form in *Chlamydomonas*, extensive invaginations like those shown in Figures 20 and 21 do form near the membrane attachment area at this time. Another possibility is that the bouquet arrangement disperses due to detachment of the bivalent ends from the nuclear envelope. Although nuclear envelope attachments were observed during zygotene and early pachytene, none were observed in the reconstructed nuclei, but most ends did remain within .2 μ of the nuclear envelope. Detachment in conjunction with pouch formation could also be the cause of dispersal.

The result that 18-20 bivalents exist per nucleus is consistent with the linkage data of Hastings *et al* (1965) concerning *Chlamydomonas reinhardi*,which found 16 linkage groups with centromeres and several fragments. These re-

sults meet the requirement for consistency with the linkage group data, that being the number of chromosomes must be equal to or greater than the number of centromer-associated linkage groups.

Structures similar to the "recombination nodules" described by Carpenter (1975a) were found associated with the pachytene bivalents of *Chlamydomonas*. The size distribution and their interaction with the bivalents is unlike the situation reported in *Drosophila*, but relate directly to her hypothesized role for them.

Two distinct size classes of dense bodies were found (Figures 20 and 21 *vs* Figure 22). This is unlike the case in *Drosophila* where a complete range of sizes was observed. It is therefore difficult to say that these two size classes are, in fact, the same structures in *Chlamydomonas*. In addition, the association of two bivalents with one spherical body is not consistent with the hypothesized relationship between them and crossing-over (Carpenter, 1975a). She claims a one-to-one correspondence between crossing-over and "nodule" associations, each nodule being associated with a single cross-over. Another observation incompatible with Carpenter's hypothesis is that two recombination nodule-like structures only .2 μ apart were found associated with a single bivalent. This is not consistent with the pattern predicted due to the interference of one cross-over event with another.

Another point unrelated to the observations made but

worth consideration concerns the fact that a significant proportion of conversion events are not associated with recombination of outside markers. If nodules were associated with both types of event, the predicted distribution would be significantly different in that non cross-over events do not interfere with cross-over events.

Due to the incompleteness of the *Chlamydomonas* linkage data, it is not possible to relate map length and number of nodes plus spherical bodies to see if there is a close correlation between the number of nodes and the expected number of cross-overs.

The stage maximum and duration of pachytene in the arginine strain were 7.9 hours and .92 hours respectively. The wild type strain yielded 9.4 hours as the stage maximum and 1.1 hours as the stage duration. The difference in length of pachytene, 0.2 hours between the arginine requiring strain and the wild type strain, can be accounted for as was done for zygotene. The difference in the stage maximum of 1.5 hours obtained when comparing probit analysis values still reflects the difference in time of entry into meiosis.

The results of the FUdR pulses indicate that a peak of increased recombination is obtained when *Chlamydomonas* is pulsed between 7½ and 8 hours after the onset of germination. This pulse period coincides with the stage maximum for pachytene, which is 7.9 hours by probit analysis. Due to the degree of synchrony and duration of pachytene, 60% of

the zygospores are in pachytene (Figure 59c) at this time.

As mentioned earlier, many experiments using chiasmata frequency as an indication of recombination levels, have suggested late zygotene-pachytene as the latest stage when recombination levels can be affected by perturbing agents. It was also mentioned that such experiments would not permit the detection of effects later than pachytene, and that only the experiments reported by Lu with *Coprinus* (Lu, 1970) would be able to detect changes in recombination frequencies occurring later than this. This is because Lu used recombination between genetically linked loci to monitor the effects of temperature and gamma irradiation on recombination. The assignment of the time of crossing-over is based upon the assumption that the latest time at which recombination levels can be altered must be a time prior to the completion of recombination events. Thus treatment of synchronous meiosis with many agents should push the estimate of the latest time when recombination levels can be altered towards its limit. Similarly, treatment with one agent or only a few agents has the weakness that the time when recombination events are completed is insensitive to the agent or agents used. It therefore seems important to test several agents known to affect recombination levels on a synchronous meiotic system. The FUdR treatment reported above was carried out because the second FUdR effect coincided with or was later than the latest time

at which several other agents could affect recombination in *Chlamydomonas*. These agents, which were tested by Chiu (1973), include mitomycin C, nalidixic acid, phenethyl alcohol, hydroxyurea, actinomycin D, cycloheximide, adenine, caffeine and acriflavine. These results (Chiu, 1973) add considerable strength to the argument that the FUdR effect at pachytene does, in fact, represent the latest stage when recombination levels can be altered and therefore shows the time when recombination events are fixed.

As *Chlamydomonas* proceeds through pachytene the chromatin becomes less condensed and the lateral element disappears. Following pachytene is a stage when the chromatin is very diffuse and bivalents are very difficult to detect (Figures 33 and 34). This lack of condensation may indicate that diplotene is a period of active transcription in *Chlamydomonas*.

The general morphology of this diffuse stage resembles that of *Coprinus* (Lu, 1965). Indicated in Figure 34 are two regions (in the bivalent in the upper right hand portion of the nucleus) which may be chiasmata. Configurations such as these are the closest things to chiasmata observed.

The stage maximum and stage duration of this diffuse stage for the arg-1 and arg-2 heterozygous strain were 8.7 hours and .51 hours, and for the wild type strain were 10.2 hours and .44 hours. Thus the duration of the diffuse stage is about ½ hour in both strains, the difference in stage maximum merely reflecting the later start in the wild

type strain.

Because of the short duration of the late prophase I, metaphase I, anaphase I and telophase I stages, these stages were lumped together when calculating stage duration, stage maxima and synchrony. Due to the lack of perfect synchrony and short durations it was not possible to sequence the stages according to their order of appearance when following samples through meiosis. Instead it was necessary to evoke a second means, this being analogy to other organisms.

Although all pachytene cells examined by serial section contained a single basal body pair, a few pachytene cells were observed with a closely associated group of basal bodies, indicating recent duplication. This, in conjunction with the fact that two basal body pairs removed some distance from one another were regularly seen in late prophase I cells and all 5 metaphase cells examined in serial section contained two pairs of basal bodies, points to late prophase as the time of their duplication in preparation for the first meiotic division.

The nucleolus begins to break up late in prophase (Figures 36 and 37) in preparation for metaphase I. This may be required to enable first division segregation to occur. A comparison of Figures 33 and 34, which represent typical diffuse stage cells, and Figures 35, 36 and 37, which represent late prophase (diakinesis) shows the increase in the degree to which the chromatin has condensed.

It also shows that the bivalents are in a much shortened form. This much shortened state is also typical of late prophase I in many organisms (Lewis and John, 1963).

Similar to the case in the vegetative division of most green algae, the meiotic spindle of *Chlamydomonas* was intranuclear. The presence of basal bodies adjacent to the polar fenestrae inplies that basal bodies are involved in microtubule organization in the meiotic divisions of *Chlamydomonas*. This observation is not surprising in light of the observation of Coss (1974) that *Chlamydomonas* vegetative cells possess basal bodies at both poles of the mitotic metaphase nucleus. It is, however, in conflict with Cavalier-Smith's claim (1974) that basal bodies are not regularly found at the poles of meiotic metaphase nuclei.

Irregularity in the shape of early metaphase I nuclei is similar to that seen in *Ulva* (Bråten and Nordby, 1973). The nuclear membrane remains intact through both divisions except for polar fenestrae. The lack of nuclear membrane breakdown is similar to the case in *Ulva* (Bråten and Nordby, 1973) and unlike that in *Lithodesmium* (Manton *et al*, 1970a) where the nuclear membrane is broken down by metaphase.

The smoother crescent shape of the nucleus later in metaphase I is similar to that observed in *Chlamydomonas moewusii* vegetative cells by Triemer and Brown (1974) and suggested by Pickett-Heaps (1973) for *Tetraspora* vegetative cells.

As the cell proceeds through metaphase and into anaphase, the nucleus becomes less hooked. It does not, however, become noticeably longer. By telophase the nucleus is no longer a hooked crescent. Instead, both ends, which are the forming daughter nuclei, are spherical in shape. Unlike the case in some protists (Floyd *et al*, 1972; Løvlie and Bråten, 1970; Stevenson, 1972) chromosome separation is accomplished by the shortening of chromosomal microtubules and not by an increase in the pole-to-pole length of the nucleus. Late in telophase the daughter nuclei are reformed and the remnants of the parent nucleus which connected them begin to break down. Similar to the conclusions of Johnson and Porter (1968) with regard to the *Chlamydomonas reinhardi* vegetative cycle products, it appears that both first division products rotate through 90° relative to the parent cell's axis. These rotations are not in the same plane.

As the zygospores proceed into metaphase I the chromatin aggregates on the metaphase plates. This aggregation is such that individual bivalents are difficult to distinguish. As metaphase I passes into anaphase I, the chromatin condenses into discrete units each with a pair of kinetochores. The occurrence of discrete kinetochores complements the genetic evidence for their existence (Ebersold *et al*, 1962). The kinetochores of first division bivalents are quite striking, resembling the kinetochores described by Pickett-Heaps and Fowlke (1970) in *Oodogonium*.

At telophase the chromatin of each forming daughter nucleus lies in a condensed sphere filling the confines of the nuclear envelope (Figure 48).

The stage duration estimates for late prophase and the diad stages were obtained from the probit analysis, due to the inability to collect complete sets of data for these stages (for reasons given earlier in the Discussion). The stage maximum was 9.1 hours and the stage duration was .46 hours for the strain heterozygous for the two arginine requirements. The stage maximum and duration for the late prophase I to telophase I stages were 10.7 hours and .43 hours for the wild type strain. The stage maxima for both strains agree if one takes into account the late entry into meiosis of the wild type zygospores. The duration of these stages is almost the same for both strains. This short duration, coupled with the amount of asynchrony, make it difficult to find samples of the individual stages within this group.

The stage classified as diads includes cytokinesis, interkinesis, metaphase II, anaphase II and telophase II. Again, these stages were treated as a single stage due to the fact that their short durations made it difficult to make many observations of any of these stages individually except interkinesis.

Cytokinesis microtubules running parallel to the cleavage furrow and radiating out from a pair of basal

bodies in each of the forming meiosis I products are shown in Figure 51. This, in conjunction with the intimate association between basal bodies and the first and second division spindles, suggests that basal bodies in *Chlamydomonas* are involved in microtubule organization. If as Heywood and Magee (1976) suggest, their presence at the spindle poles is just to ensure their own segregation to each product, it seems unlikely that they would be associated with cytokinetic microtubules as well. The association of microtubules with the cleavage furrow (Figures 50 and 51) suggests that microtubules are involved in cytokinesis during meiosis as well as mitosis (Johnson and Porter, 1968).

The occurrence of a diffuse interphase suggests that active transcription and translation occurs. This is further supported by the reformation of the nucleolus (Figures 51 and 53). The nucleolus disappears for metaphase II, the chromosomes again have discrete kinetochores. Although the second division kinetochores resemble the first division ones in general morphology, they are not as large as the first division kinetochores (Figure 45 *vs* Figure 55). This may be the result of the kinetochores of each homolog fusing in the bivalents while being separated to facilitate chromatid separation in the second division.

Owing to the incompleteness of the data it was only possible to estimate stage maximum and duration of the diad stage by probit analysis. The probit analysis gave

9.6 hours and .52 hours for the arginine requiring strain and 11.1 hours and .45 hours for the wild type strain. Again, the stage maxima are similar taking into account the 1.5 hour delay in entrance into meiosis of the wild type strain, and the stage durations are also similar.

Figure 57 shows that the nuclei return to an interphase state with a single nucleolus prior to completion of the cleavage furrows yielding the four discrete meiotic products.

The degree of synchrony varies from 2.6 hours for pachytene to 3.6 hours for zygotene in the strain heterozygous for the two arginine requirements, and from 3.5 hours for diads to 4.5 hours for diplotene and late prophase I through telophase I for the wild type strain. The general trend is not simply a decrease in synchrony with time. Instead, the degree of synchrony of both strains improves as they enter pachytene. There are two non-trivial explanations for this. The first is that the zygospores require the formation of a fixed level of metabolite before entering pachytene. The second is that they communicate. The high degree of synchrony within zygospore clumps (personal observation) suggests that communication may occur.

Earlier it was mentioned that *Chlamydomonas* provides a nearly ideal system for the study of meiosis, the only serious deficiency being that very little was known about the cytology. This made it difficult to justify its use

as an organism for the study of meiosis because it was not known what cytological aspects of meiosis in *Chlamydomonas* were typical of those observed in other organisms. The work presented here has shown that the chromosomal events occurring during meiosis in *Chlamydomonas* are typical of those found in most organisms (Gillies, 1975a). In addition, it was possible to define the maxima and duration of the different cytological stages.

Having established the typical nature of meiosis in *Chlamydomonas*, it was possible to show that the DNA synthetic period in preparation for the meiotic divisions occurred just prior to prophase I. Conclusive evidence that recombination events are not completed prior to pachytene was presented. In addition, very suggestive evidence that recombination occurs at pachytene was obtained. This is particularly compelling in conjunction with the results of Chiu (1973). The significance of this observation lies in the fact that these are the only extensive studies using a system which could determine the time of meiotic crossing-over if it occurred after pachytene.

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